The ionic balance of the sugar-beet plant

F. van Egmond

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Aan mijn ouders

Dit proefschrift met stellingen van Frans van Egmond, landbouwkundig ingenieur, geboren te Gouda op 31 juli 1941, is goedgekeurd door de promotoren, dr. A.C. Schuffelen, hoogleraar in de landbouwscheikunde en dr.ir. C.T. de Wit, buitengewoon hoogleraar in de theoretische teeltkunde.

De Rector Magnificus van de Landbouwhogeschool
H.A. Leniger

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Proefschrift
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doctor in de landbouwwetenschappen,
op gezag van de rector magnificus, dr.ir. J.P.H. van der Want,
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Abstract

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The ionic balance of the sugar-beet plant was studied by measuring dry weight and chemical composition of every leaf, the crown and the root during the growing season.

The sugar-beet plant has an almost neutral uptake. The nitrate and sulphate reduction determines the amount of carboxylates in the plant. Accumulation of carboxylates prevents large changes of cell-pH because of the ion-uptake and ion-utilization processes.

In every plant part the carboxylates are a quantitative measure of the amount of nitrate reduced. It is concluded that nitrate is mainly reduced in the leaves and to a small extent in the root.

It is proved that for the sugar-beet plant, Cl can be used as an index element to calculate the supply of the various ions to the various plant parts. Redistribution of potassium, calcium, magnesium, phosphate and organic N is determined by comparing the actual and calculated amounts in the various plant parts. The redistribution of potassium from the old leaves to the root is counterbalanced by redistribution of divalents in the opposite direction. The redistribution of the divalents takes place from organs with a relatively small oxalate production towards organs with a relatively large oxalate production.

In an appendix a rapid carboxylate determination is described.

This thesis will also be published as Agricultural Research Reports 832.

Stellingen

1. De hoeveelheid carboxylaat (≈ C-A) en de in de loop der tijd gereduceerde hoeveelheid nitraat zijn in alle organen van de suikerbietenplant bij benadering gelijk aan elkaar.

Dit proefschrift

2. De redistributie van organisch stikstof over de organen van de suikerbietenplant kan berekend worden uit het verschil C-A en de hoeveelheid organisch stikstof per orgaan.

Dit proefschrift

3. De redistributie van natrium, kalium, calcium, magnesium, fosfor en stikstof binnen de suikerbietenplant kan berekend worden met chloor als index-element.

Dit proefschrift

4. Nitraat wordt in de suikerbietenplant niet geredistribueerd.

Dit proefschrift

5. Redistributie van calcium en magnesium binnen de suikerbietenplant vindt plaats van organen met een relatief geringe oxalaatproduktie naar organen met een relatief grote oxalaatproduktie.

Dit proefschrift

6. Uitputting van de nitraatvoorraad in augustus bevordert de kwaliteit van de suikerbiet.

R.S. Loomis & D.J. Nevins, J. Am. Soc. Sugar Beet Techn. 12 (1963), p. 309-322
Dit proefschrift

7. Het is aannemelijk dat naast het door Dijkshoorn voorgestelde mechanisme ook andere mechanismen van belang zijn bij de redistributie van kalium.

E.A. Kirkby, Proceedings of the 7th international colloquium on plant analysis and fertilizer problems, volume 2 (1974), p. 557-568

8. Nitraatreductase kan behalve door nitraat ook door andere stoffen, zoals citroenzuur, geïnduceerd worden.

A.R. Ferguson & J.S. Knypl, Proceedings of the 7th international colloquium on plant analysis and fertilizer problems, volume 1 (1974), p. 101-109

9. Dat Marschner en medewerkers bij ijzergebrekkige zonnebloem wel, en bij ijzergebrekkige maïs geen pH-daling in de voedingsoplossing waarnemen, komt omdat maïs, in tegenstelling tot zonnebloem, onder normale omstandigheden veel meer anionen dan kationen opneemt.

H. Marschner, A. Kalisch & V. Römheld, Proceedings of the 7th international colloquium on plant analysis and fertilizer problems, volume 2 (1974), p. 273-281

10. Tot de door van Goor en Wiersma veronderstelde aanwezigheid van ionogeen calcium in zeefvaatsap mag niet worden geconcludeerd op basis van hun proefresultaten.

B.J. van Goor & D. Wiersma, Physiol. Plant. 31 (1974), p. 163-168

11. De audio-visuele middelen worden in het wetenschappelijk onderwijs in vele opzichten ondoordacht ingevoerd en toegepast.

12. De studierichting bodemkunde en bemestingsleer aan de Landbouwhogeschool heeft in haar verplichte kandidaatsprogramma te weinig plaats ingeruimd voor de microbiologie.

13. De weergave van het openbare optreden van Jezus in Jesus Christ Superstar komt overeen met de zienswijze van Albert Schweitzer.

A. Schweitzer, Geschichte der Leben Jesu Forschung (1906)

14. Het verdient aanbeveling om in concert- en toneelzalen faciliteiten te scheppen voor voldoende zeer goedkope staanplaatsen.

F. van Egmond
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Voorwoord

Bij het beëindigen van deze studie maak ik gaarne van de gelegenheid gebruik, allen, die op enigerlei wijze bijgedragen hebben aan dit werkstuk, mijn dank te betuigen.
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- Tenslotte dank ik Alice, Frederik Frans, Mirjam en Robert-Jan voor alle afleiding.

Curriculum vitae

Na het behalen van het diploma HBS-B aan het Christelijk Lyceum in Rotterdam begon de auteur in 1959 zijn studie aan de Landbouwhogeschool te Wageningen. Hij specialiseerde zich in de bodemkunde en bemestingsleer. Tijdens zijn studie werd hij in 1967 aangesteld op de afdeling Landbouwscheikunde van de Landbouwhogeschool. Na zijn afstuderen in datzelfde jaar werd hij opgenomen in de wetenschappelijke staf van voornoemde afdeling.
In de periode 1969-1970 was hij in een aantal functies actief bij de herprogrammering van de studierichting bodemkunde en bemestingsleer. Na de herprogrammering was hij achtereenvolgens studiecoördinator van voornoemde studierichting en in de periode 1972-1974 secretaris van de Vaste Commissie voor het Onderwijs van de Faculteit der Landbouwwetenschappen.

Contents

1 Introduction

In the 1960s, Dutch scientists introduced the (C-A) concept. This is the difference between inorganic cations (C) and inorganic anions (A). It arises from the uptake, utilization and redistribution of ions within the plant. As the rate of dry matter production is mainly determined by the amount of ions taken up, utilized or redistributed, a relation between (C-A) content and yield might be direct or indirect and partly reflect the effect of the ionic balance on the yield.

At the Department of Soils and Fertilizers, Agricultural University, Wageningen, a study on the ionic balance of the sugar-beet was started in 1969 (van Egmond et al., 1970; Houba et al., 1971; van Egmond, 1971).

It was known that sugar-beet plants contain more (C-A) per kg dry matter than for instance the graminae. The sugar-beet plant appeared to produce about as much (C-A) as it reduces nitrate, when grown on culture solutions with nitrate as nitrogen source. However in most leaves the (C-A) content and the organic-N content differ largely. This raised some questions for further research. Is the (C-A) production equal to the nitrate reduction of sugar-beet plants grown in the field when nitrate is the nitrogen source? Is the difference between the (C-A) content and the organic-N content of the plant parts a result of redistribution of organic N, (C-A) or both?

Redistribution is often studied in short-term experiments with the aid of tracer techniques and often with parts of plants. It would be interesting to know whether a quantitative picture of the redistribution process within the sugar-beet plant can be obtained by regularly harvesting plants in a long-term experiment and analysing the various plant parts.

With nitrogen fertilization it is known that the (C-A) production diminishes as soon as the nitrate pool in the plant is depleted. The nitrate reduction stops but due to alkaline uptake the plant still produces some (C-A) in this situation. And this leads to the question whether the quantitative picture of the redistribution process differs for sugar-beet plants fertilized with a low or a high amount of nitrate.

The final composition of the sugar-beet crop - with its long growing

period - is of great importance, as the quality of sugar-beet roots is largely determined by chemical characteristics such as the sugar, sodium, potassium and noxious nitrogen content. Ion uptake and the redistribution of nutrients affect these chemical characteristics. Noxious nitrogen especially depresses root quality and therefore I studied how much and from where the organic N is redistributed towards the root during the growing period. From the differences in the redistribution of organic N between the nitrate treatments I tried to determine how nitrogen should be fertilized for an optimum root quality. Another question connected with quality, is how much and from where cations are redistributed towards the root during the growing period. It is known that potassium is redistributed towards the root and that the redistributing cations should be accompanied by anions, but which anions are redistributed and what is the role of the carboxylates in the cation redistribution process? In general, which cultivation practices that affect redistribution, improve root quality?

2 Literature

2.1 (C-A)

2.1.1 Terminology

Normally the total amounts of positive and negative charge per unit of plant material are equal. Excess positive as well as negative charge only occurs here and there in the electroneutral plant material.

The elements K, Na, Ca and Mg are mainly present as ions and the amount of positive charge carried by minor elements and organic cations is relatively small. Therefore the total amount of positive charge in plant material, C is approximately equal to: $K^+ + Na^+ + Ca^{2+} + Mg^{2+}$ equivalents (de Wit et al., 1963).

The negative charge is carried by the inorganic anions Cl^-, NO_3^-, $H_2PO_4^-$, SO_4^{2-} and organic anions, such as citrate, malate and oxalate. The total amount of inorganic negative charge in plant material, A is approximately equal to: $Cl^- + NO_3^- + H_2PO_4^- + SO_4^{2-}$ equivalents.

The amount of charge, which is not carried by inorganic ions, is given by the difference (C-A). The apparent excess of charge, represented by (C-A), is neutralized by organic anions (Arnon, 1939; Ulrich, 1941 and 1942; Pierce & Appleman, 1943; Jacobson & Ordin, 1954). The amounts of free organic acids present in the plant material are, at pH values exceeding 5, relatively small.

If in the calculation of A the $H_2PO_4^-$ value is based on both inorganic ($H_2PO_4^-$) and organic ($RHPO_4^-$) phosphorus the (C-A) refers to the salts of carboxylic acids only (van Tuil, 1965). The term carboxylates is then preferred.

2.1.2 Processes

Generally there is no paired uptake of C and A from neutral salts (Mayer, 1920; de Wit et al., 1963). Processes active during ion uptake and ion utilization cause the positive (C-A) in plant material. Table 1 gives these processes and their influence on the carboxylate pool.

When there is a substantial uptake of ions other than those summed up in

Table 1. Processes influencing the carboxylate pool (Houba et al., 1971).

Process	Carboxylate
1. Uptake $Na^+ + K^+ + Ca^{2+} + Mg^{2+}$ meq < uptake $NO_3^- + Cl^- + SO_4^{2-} + H_2PO_4^-$ meq	decrease
2. Uptake $Na^+ + K^+ + Ca^{2+} + Mg^{2+}$ meq > uptake $NO_3^- + Cl^- + SO_4^{2-} + H_2PO_4^-$ meq	increase
3. Nitrate reduction	increase
4. Sulphate reduction	increase
5. Transition of NH_4^+ into organic N	decrease

the Processes 1 and 2, such ions, for instance NH_4^+, should be considered too.

During ion uptake electroneutrality is maintained. Yet, there are differences in amount of C and A uptake as shown in the Processes 1 and 2. This is the result of influx and efflux of H^+, OH^-, HCO_3^- over the boundary between root and outer solution. The amount of charge which causes the root potential is very small.

Ulrich (1941) established that uptake of more A than C is accompanied by a decrease in the amount of carboxylates present in the absorbing tissue. It is then assumed that electroneutrality is maintained by efflux of HCO_3^- (Hoagland & Broyer, 1936; Arnon, 1939; Coïc, 1961) or by uptake of H^+ (van Tuil, 1965; Dijkshoorn et al., 1968). In Fig. 1 both possibilities are represented.

Plants can take up excess A continuously, when due to other processes

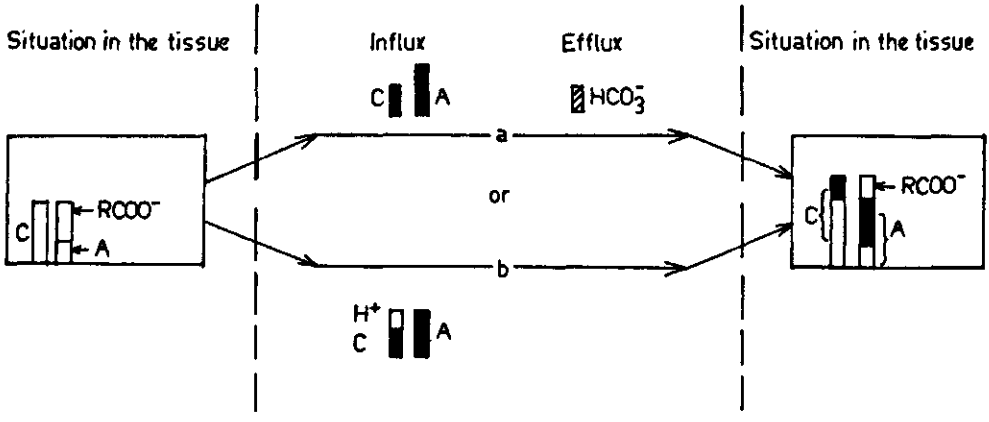


Figure 1. Schematic representation of acidic uptake.

(e.g. Process 3) carboxylates are produced in or transported to the roots. Dijkshoorn et al. (1968) uses this possibility to interpret the results obtained with perennial ryegrass grown on nitrate as nitrogen source.

When a tissue takes up more C than A, a simultaneous increase of carboxylates may occur (Ulrich, 1941 and 1942). Uptake of OH^- or HCO_3^- (van Tuil, 1965; Dijkshoorn et al., 1968) or efflux of H^+ (Jacobson & Ordin, 1954; Poole & Poel, 1965) maintains the electroneutrality. Both possibilities are represented in Fig. 2. According to de Wit et al. (1963), at least in balance studies, the distinction between exchange with H^+ and paired uptake with HCO_3^- is rather academic.

Alkaline uptake without a simultaneous increase of carboxylates may occur when nitrogen is taken up as NH_4^+, because the effect of Process 2 is opposite to that of Process 5.

Direct evidence shows that, when bases enter the cell in association with nitrate and the latter is subsequently reduced, the basic residue is neutralized by organic acids (Hoagland & Broyer, 1936). The following equation is given by Dijkshoorn (1962, 1964) for Process 3 of Table 1:

$$(NO_3)^- + 8\ H^+ + 8e \rightarrow (NH_3) + 2\ H_2O + OH^-$$

Nitrate reduction and carboxylate production of sugar-beet plants grown on nitrate as nitrogen source are almost stoichiometrically interrelated, as is shown in Table 2.

In plants sulphate reduction too is accompanied by carboxylate production. According to Dijkshoorn & van Wijk (1967), organic S is about 6% of organic N

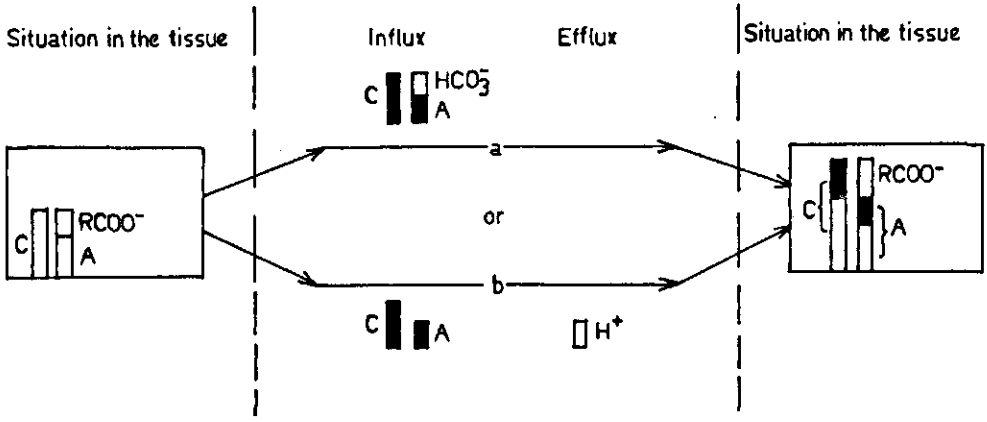


Figure 2. Schematic representation of alkaline uptake.

Table 2. Organic N and (C-A) in young sugar-beet plants.

van Egmond & Houba, 1970			Houba et al., 1971		
Days after germination	Organic N in mmole/plant	(C-A) in meq/plant	Days after germination	Organic N in mmole/plant	(C-A) in meq/plant
26	4.65	4.50	30	8.10	8.26
30	5.86	5.60	36	21.4	21.3
37	10.1	10.6	39	25.5	26.3
40	13.1	14.0	43	26.1	26.7
42	22.6	24.3	46	42.9	44.3
			51	45.6	47.3

on equivalent basis. If the (C-A) values given in Table 2 are to be explained by Processes 3 and 4 only, the substraction (C-A) - (Organic N) gives the amount of sulphate reduced (meq per plant). From all values of Table 2 an average value of about 3.6% is calculated for organic S to organic N on equivalent basis. This value is of the right magnitude.

Plants grown on NH_4^+ as nitrogen source, have a relatively low content of carboxylates (Clark, 1936; Arnon, 1939; Chouteau, 1960 and 1963; Coïc et al., 1961; de Wit et al., 1963; van Tuil, 1965; Jungk, 1967; Houba et al., 1971). The presence of carboxylates in plants grown on NH_4^+ as the only source of nitrogen is mostly due to operation of Processes 2 and 4, while Process 5 causes the carboxylate pool to decrease. In Fig. 3 the overall effect of uptake and utilization of NH_4^+ is given schematically.

Changes that occur in the carboxylate pool prevent great fluctuations in pH of the cell solution due to ion uptake and utilization (Hoagland & Broyer, 1936; Ulrich, 1941; Chouteau, 1960; Coïc, 1961; de Wit et al., 1963; Dijkshoorn, 1964; van Tuil, 1965). Accumulation of carboxylates increases the buffer capacity of the cell solution.

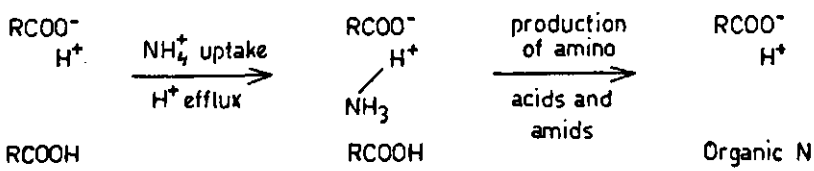


Figure 3. Schematic representation of the influence of NH_4 uptake and utilization on the carboxylate pool.

2.1.3 Distribution of carboxylates

When plant parts are arranged according to increasing cation content, the sequence is mostly roots, stems, leaves and the C content increases from young to old leaves. The accumulation of C is generally explained by the accumulation of Ca^{2+} in the old leaves, while Mg^{2+} accumulation is also of importance. The K^+ contents of the various plant parts differ relatively less. Sometimes accumulation of Na^+ in the leaves is reported but in many experiments Na^+ is not determined. Compared to C, the amount of Na^+ in the plant is small, except in some plants e.g. sugar-beet and spinach, that have preference for Na^+ with respect to the uptake of Na^+ and K^+. Some grasses - dressed with high amounts of Na^+ - containing fertilizers - can take up much Na^+.

Although the NO_3^- and Cl^- contents especially in stems and the $H_2PO_4^-$ content in young aerial parts can be high, A and C do not accumulate stoichiometrically. By the operation of Processes 3 and 4 of Table 1, inorganic salts are replaced by carboxylates.

Well fertilized plants in which Processes 1, 2 and 5 are hardly active, take up C and A almost stoichiometrically. If the average organic-N content is assumed to be about 2.5 mole per kg dry matter, an organic-S content corresponding with about 0.15 eq SO_4^{2-} per kg dry matter is calculated (Dijkshoorn & van Wijk, 1967). The carboxylate content will then be about 2.5 + 0.15 = 2.65 eq per kg dry matter. Assuming C uptake to be about 3.5 eq per kg dry matter produced, the A content is about 3.5 - 2.65 = 0.85 eq per kg dry matter. In this representative example, there are 3 times as much carboxylates as inorganic salts; as a rule of thumb it is often said that inorganic cations and carboxylates accumulate to about the same extent (Chouteau, 1960; Coïc, 1962, 1963a, 1963c and 1969; Mengel, 1965; Kirkby & deKock, 1965; Bengtsson et al., 1966; Houba et al., 1971).

A good correlation between Ca^{2+} and oxalate was reported by Olsen (1939), and Coïc (1963); a strong relationship between insoluble Ca^{2+} + Mg^{2+} and insoluble oxalate in spinach was reported by Bengtsson (1966). Others report lack of relationship between Ca^{2+} and oxalate (Coïc, 1969) or a relationship between Ca^{2+} and malate (Kirkby & deKock, 1965). When a relation is found between a cation and oxalate or malate it does not necessarily mean that the accumulation of the cation is caused by oxalate or malate.

When plants such as quayule (Cooil, 1948), tobacco (Chouteau, 1960), tomato (Kirkby, 1969), sugar-beet (van Egmond & Houba, 1970) and possibly spinach (Bengtsson et al., 1966) are well nourished only Processes 3 and 4 are

active. Then the carboxylate production is stoichiometrically equal to the reduced amount of nitrate (+ sulphate). If the nitrate reduction is mainly located in the leaf apparatus and the carboxylates accumulate where they are made, then the amount of carboxylate is equivalent to the amount of nitrate reduced in the leaf under consideration (Chouteau, 1960; Coïc, 1962; Kirkby & deKock, 1965; van Egmond, 1971).

Mostly the amount of eq carboxylates per leaf and the amount of organic N expressed as eq NO_3^- reduced differs and it is concluded from these differences, that a part of the organic N produced in old leaves is redistributed (Chouteau, 1960; Coïc, 1962, 1963a, 1964 and 1969; Kirkby & deKock, 1965; van Egmond, 1971).

2.1.4 The normal (C-A)

According to de Wit et al. (1963), there is much variation in the C and A contents in plant material of one plant species, but the (C-A) content hardly varies. However, the (C-A) content of different plant species and of various plant parts may differ considerably. De Wit supposed that the growing plant regulates the (C-A) content within narrow boundaries - known as the normal (C-A) content - as and when the nutrition of the plant is not disturbed. Various normal (C-A) contents have been determined for different plant species (de Wit et al., 1963; van Tuil, 1965; Jungk, 1967; Dijkshoorn et al., 1968; Ismunadji & Dijkshoorn, 1971), while Arnold Bik (1970) indicated an optimum (C-A) content for gloxinia.

It is doubtful whether the normal (C-A) concept is of general use, because the (C-A) content of plants decreases during the growing period (see Table 3) and because the (C-A) content of sugar-beet leaves for instance may range from about 1.5 up to 6 eq per kg dry matter (van Egmond, 1971).

Table 3. The (C-A) content of young sugar-beet plants (van Egmond & Houba, 1970).

Days after germination	The weighted average (C-A) content of the whole plant in meq per kg dry matter.
30	3600
36	3000
39	2940
43	2860
46	2520
51	2670

Somewhere between the root surface and the vascular tissue there is a barrier to the free movement of ions. They are actively absorbed by the plasmalemma and, in competition with active absorption by the tonoplast, they move passively towards the xylem where a leaky stelar parenchyma is the linchpin of the symplasm theory of radial transport (Laties, 1969).

An aqueous solution of nutrients is transported through the xylem. This transport is caused by the difference in water potential along the xylem track. Generally the rate of transport is mainly determined by the transpiration, which is influenced by the hydraulic properties of the soil, the soil water content, the depth of penetration and the concentration of the roots, and the internal conductances of root, stem and leaves (Milthorpe & Moorby, 1969). Transpiration rates from 0 up to about 7 mm per day have been recorded.

Transport of ions through the xylem is to some extent influenced by precipitation of insoluble compounds (Gauch, 1957; Bollard, 1960) and by the ion uptake of the cells adjacent to the xylem.

The concentration of inorganic ions in the xylem sap is about 400 - 2500 µg per ml (Milthorpe & Moorby, 1969) and mostly higher than the concentration in the nutrient solution.

Because of the following observation, there is no unanimity about the transport process in the phloem: -locally applied tracers can rapidly move in two directions; they are detected in stylet exudates both above and below the point of application of the tracer -together applied tracers are transported at different rates - the movement in the phloem can change of direction.

Speeds in the phloem of 10 - 100 cm per hour have been reported (Zimmermann, 1960), while Canny (1971) expressing the transport rate in amounts of dry matter transported per cm^2 cross-section of the phloem, gave 0.4 - 4 gram per cm^2 per hour.

More than 40% of the solved material transported by the phloem are carbohydrates (Zimmermann, 1960); the concentration in exudates ranges from about 10 - 25 gram per 100 cm^3.

From the recovery of foliar-applied radioactive isotopes, Bukovac & Wittwer concluded that Rb, Na and K are mobile; P, Cl, S, Zn, Cu, Mn, Fe and Mo are in an intermediate position; while Ca, Sr and Ba are taken up by, but are not transported out of the leaf. The explanation for the last observation is generally sought in the processes which control the entrance into the sieve tubes, as Ca is rapidly transported the moment it enters the phloem.

Table 4. Export of N and P at four nitrogen levels from the leaves of wheat as percentage of the maximum value (Williams, 1955).

Treatment	N_1	N_2	N_3	N_4
Percent export of N	84	86	86	82
Percent export of P	83	91	90	89

The concentration of nitrogen in exudates of the sieve tube ranges from about 0.03 - 0.4 gram per 100 cm^3. It is mainly transported as organic N (Zimmermann, 1960). Phosphates are present in concentrations up to about 0.1 gram per 100 cm^3. The phosphates in the exudates, gathered from aphidian stylets are mainly organic phosphates (Esrich, 1970). The potassium concentrations in sieve tube exudates may be very high. De Wit et al. (1963) assumed that potassium and carboxylates are transported through the phloem.

Redistribution of the accumulated nutrients and their derivatives enables prolonged growth and dry matter production. Nutrients that are neither consumed nor accumulated at the endings of the xylem system are redistributed through the phloem, but the vascular connections appear to be more extensive and not to influence translocation as much as previously thought (Milthorpe & Moorby, 1969).

An example of nitrogen and phosphate redistribution from wheat leaves is given in Table 4.

The general view is that the growth of the primordial leaf fully depends on the import of all basic materials from other plant parts, such as sugars, amino acids and phosphate. In the next phase of development, which can last until the leaf reaches the maximum surface, these materials are both imported and exported whilst sugar is already net exported and nitrogen is still net imported. In the last phase of development there is net export from the leaf of sugars, nitrogen, potassium and phosphate in which phase the capacity of the exporting system is maintained until the leaf is in an advanced state of senescence.

3 Material and methods

3.1 FIELD TRIAL

At Wageningen on one of the trial fields of the Department of Field Crops and Grassland Husbandry of the Agricultural University, an experimental site was prepared on 2 May 1969. The analytical data of the light coloured, gravelly sandy soil are given in Table 5.

The following amounts of fertilizers (kg per ha) had been applied to the experimental site between the growing periods:

- 133 kg K as 400 kg muriate of potash 40% (160 kg K_2O),
- 83 kg P in the form of 600 kg of basic slag (84 kg P_2O_5) and in the form of superphosphate (108 kg P_2O_5),
- 45 kg Mg, as 200 kg of kiserite (50 kg MgO) and in the dolomitic-limestone (25 kg MgO),
- 174 kg Ca as 500 kg of dolomitic-limestone (226 kg CaO).

When the site was ready for sowing:

- 60 kg N per ha in the form of nitrate of lime,

Table 5. Soil analytical data of the experimental site.

pH-KCl	4.6	K-HCl	11
organic matter	2.7 %	K-(getal)	17
P-AL	34	MgO-NaCl	34

P-AL = ammoniumlactate-acetic acid extractable P expressed in mg P_2O_5 per 100 gram soil.

K-HCl = exchangeable K (0.1 N HCl) expressed in mg K_2O per 100 gram soil.

K-(getal) = calculated number from K-HCl and humus content.

MgO-NaCl = exchangeable Mg (0.5 M NaCl) expressed in mg MgO per kg soil.

- 20 kg of Borax per ha and
- Pyramin as pre-emergence weed killing spray, was applied.

After emergence of the plants one half of the site was top dressed with 190 kg N per ha in the form of nitrate of lime.

Diploid sugar-beet seed was drilled at a plant distance of 80 by 80 cm, 3 seeds per hole. Diploid seed, van der Haven number P 2167, was used so that results could be compared with those of Houba (1973). The experimental scheme is shown in Fig. 4.

After emergence the number of plants was reduced to one plant per hole. Owing to the wide spacing the fields had to be hoed frequently to suppress the weeds. The open stand also promoted infection by lice and the crop was therefore sprayed with Basudine at regular intervals.

The soil being rather susceptible to drought several sprinkler irrigations were applied during the sunny and dry growing period of 1969; 2 July, 30 mm; 18 July, 20 mm; 22 July, 10 mm; 28 July, 35 mm; 6 August, 30 mm.

During the growth of the crop the number of leaves was regularly counted and leaves of the same numerical order marked with coloured plastic strings.

On 3 and 17 July, 1, 15 and 29 August and 26 September 1969 ten plants, collected at random from each treatment, were harvested. Because of the wide spacing, the development of the remaining plants was not influenced. After harvesting, the plants were divided in the following sections: leaf blades, petioles, crowns and roots. Figure 5 illustrates the way of sampling. Blades of leaves of the same numerical order from 10 harvested sugar-beet plants were mixed together to form one sample. Similarly the corresponding petioles formed a sample bearing the same number as the leaf sample. Sample A consisted of ten crowns, sample B of ten roots. As on 26 September there were many leaves emerging from the axil of the earlier formed leaves, the plants of this harvest

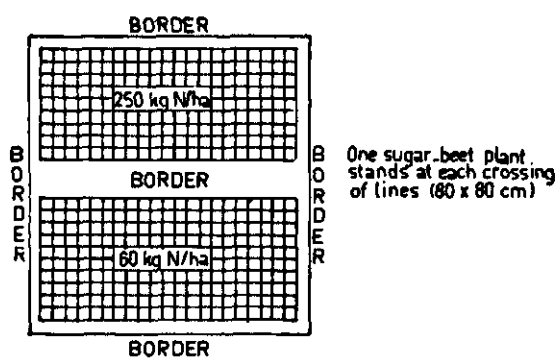


Figure 4. Scheme of trial field.

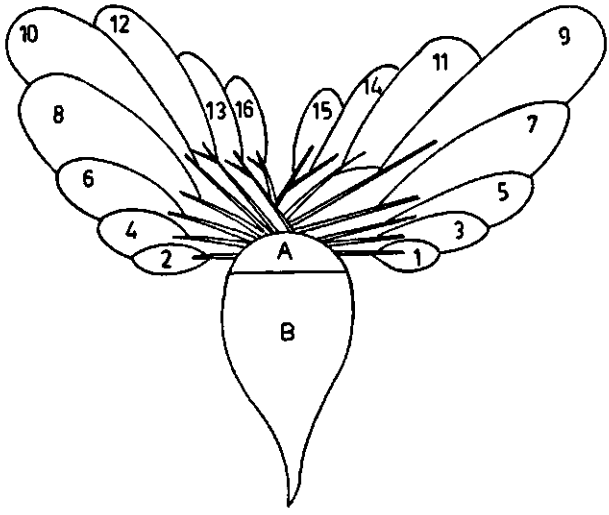


Figure 5. Position of the separate samples in the sugar-beet plant.

were only divided into the sections: leaf blades, petioles, crowns and roots, and all leaf blades and all petioles were treated together.

The root and crown samples were cut and subsamples were taken. After determining the fresh weight, all the samples were dried at 70°C for 24 hours, weighed again and finely ground for chemical analysis.

3.2 CHEMICAL ANALYSIS

Samples were analysed for Na, K, Ca, Mg, H_2PO_4, NO_3, Cl, SO_4, total N and organic acids. All values were calculated on the basis of oven-dried material (70°C).

Subsamples were analysed after digestion in concentrated sulphuric acid and hydrogen peroxide in the presence of salicylic acid. In this digest, the Na, K and Ca contents were determined by flame photometry, the H_2PO_4 content colorimetrically, the Mg content by atomic absorption and the nitrogen content by the micro-Kjehldahl procedure. Other subsamples were extracted with water (0.5 gram plant material and 50 ml deionized water: shaking time 2 hours). In this extract the nitrate content was determined with an Orion nitrate electrode, the Cl content was determined coulometrically with a chlorocounter and the SO_4 content colorimetrically. For a detailed description of these analyses see Slangen & Hoogendijk (1970). The total of carboxylates (C-A) was calculated by subtracting the sum of inorganic anions from the sum of inorganic cations

(de Wit et al., 1963).

The carboxylates were converted into organic acids with a H^+ sulphonic acid resin. These then were resolved by gradient elution partition chromatography and quantitatively determined with an automatic titrator. In the Appendix a detailed description of this method is given.

Since the treatment did not extract all the oxalates, the remaining oxalates were determined oxidimetrically with $KMnO_4$ following a double treatment of the samples with 2 N HCl. In the blank determination hardly any $KMnO_4$ was used.

4 Production and composition

4.1 INTRODUCTION

Dry matter production is easily and quickly determined and from the dry matter production curve it can be seen whether the growth of the crop is regular. This type of control is necessary to avoid mistakes in a more detailed study of the results. The observed dry matter production was too low due to losses in the field. A comparison of the losses from leaf and root material with the dry matter production gave the relative importance of these losses.

The quality of sugar-beet roots is mainly determined by the sugar content and therefore attention will be paid to it in this chapter.

As already mentioned, sugar-beet plants have a neutral uptake when grown on culture solutions with nitrate and an alkaline uptake as soon as nitrate in the plants is depleted. In this chapter the C and A uptake of sugar-beet plants grown in the field are compared, with special interest in differences between the high and low N-treatment.

Whether the positive (C-A) value in plant material is neutralized by negatively charged carboxylates is a classical question. In this chapter whole plant (C-A) values and the sum of carboxylates will be compared for additional information.

The whole plant (C-A) and organic-N values will be compared and special attention will be paid to differences between the high and low N treatment.

From literature data it is known that the decrease of (C-A) and organic-N content during the growing period is considerable. The content of a substance in the dry matter of the whole plant is determined by uptake, utilization, production and dilution with other dry matter. In this chapter the course of the various contents during the growing period are given to show the difference between the various substances and to show that redistribution cannot be studied by only comparing contents at different intervals of the growing period.

The harvested dry matter weight per plant is given in Fig. 6: these weights are calculated by summing up the weights of the various plant parts. These results are too low for two reasons.

A closed sugar-beet crop loses during the growing period about half its amount of leaf material (Houba, 1973). In my experiment losses were considerably less, as the period of highest loss of leaf material was avoided and possibly because of the absence of mutual shading. Moreover, Houba's estimations were of dead leaf material and my estimations concerned the part of the leaves that was really lost from the plants. In Table 6 the numbers of the leaves lost and an estimation of the accumulated loss is given: when the loss of a leaf was observed its weight at the previous harvest was added to the accumulated loss.

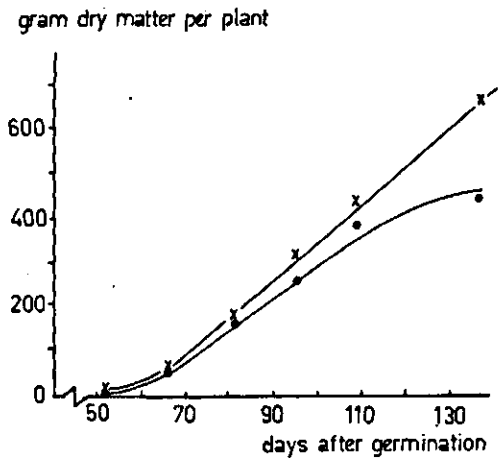


Figure 6. Amount of dry matter harvested per plant during the growing period. . = 60 kg N per ha and x = 250 kg N per ha.

Table 6. Estimation of the loss of leaf material during the growing period.

Days after germination	Leaf number of leaves lost	Accumulated dry matter loss in gram per plant	
		60 kg N per ha	250 kg N per ha
52	none	0	0
66	1, sometimes 2	0.05	0.05
81	1, 2	0.07	0.07
95	1, 2, 3	0.35	0.40
109	1, 2, 3, 4	1.10	1.40
137	1, 2, 3, 4, 5, 6, 7	4.45	4.50

From Table 6 and Fig. 6 it follows that nearly 1% of the dry matter was lost by leaf fall.

A part of the root material was not harvested, but was left behind in the soil. If the ratio of shoot to fibrous root for sugar-beet plants is known, these losses can be estimated. In experiments of van Egmond & Houba (1970) young sugar-beet plants grown in nutrient solutions had a shoot-root ratio of about 5 and van Lieshout (1956) found for some four-month-old crops ratios between 3.45 (white mustard) and 10.24 (pea). Young sugar-beet plants grown in the field had a shoot-root ratio between 10 and 15 (van Ginneken, 1945). Therefore, the estimations of the amount of root material not harvested are based on a shoot-fibrous root ratio of 10. These estimations are given in Table 7. They indicate that about 5% of the produced dry matter stayed behind in the soil. However, the shape of the curves and the distances between them are not essentially altered when Fig. 6 is corrected for the estimations given in Tables 6 and 7. Therefore, the data of the total amount of dry matter harvested per plant will be treated as if these figures were the total amounts produced.

During the whole growing period the dry matter production for the low nitrogen treatment was lowest, Fig. 6. The production of new leaf material almost ceased in the period between the last two harvests which is an indication of nitrogen shortage.

With the high nitrogen treatment, dry matter production per unit of time was almost constant between 66 and 137 days. Under favourable conditions 200 kg dry matter per ha per day can be produced by a closed crop (de Wit, 1971), but due to the wide spacing of the plants in the field, not more than 130 kg was produced in the high nitrogen treatment. The dry matter production curves show

Table 7. Estimation of the weight of root material not harvested in gram dry matter per plant.

Days after germination	60 kg N per ha	250 kg N per ha
52	1	1
66	3	4
81	7	5.5
95	11.5	13
109	14.5	18
137	15	24

Table 8. Sugar content in fresh sugar-beet sample.

Days after germination	60 kg N per ha	250 kg N per ha
95	11.0%	10.5%
109	11.7%	11.2%
137	14.0%	13.1%

that for both treatments the growth of the crop was as expected.

Sugar content was determined in samples of fresh beet of the last three harvests. The results are given in Table 8. For the low nitrogen treatment the content was always highest. This result does not mean that the sugar production per plant with this treatment was highest, as these plants produced less fresh beet per plant than with the high nitrogen treatment. These contents and the difference between the treatments are as expected at this interval of the growing period (95 - 137 days).

4.3 UPTAKE AND UTILIZATION OF INORGANIC IONS

The amount of cations taken up by the plants during the growing period is plotted in Fig. 7. With the low nitrogen treatment this amount increased regu-

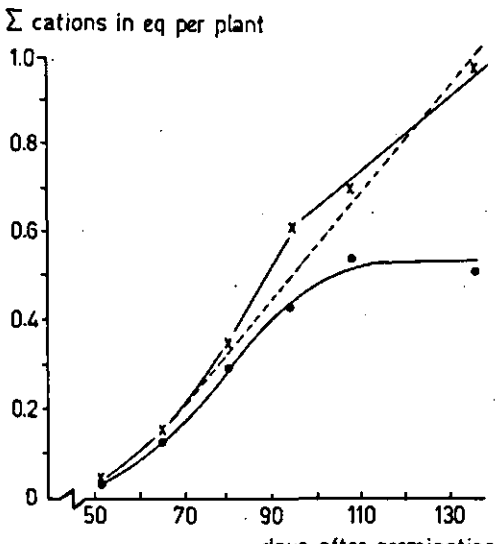


Figure 7. Amount of cations taken up by the plant during the growing period. . = 60 kg N per ha and x = 250 kg N per ha.

larly up to 109 days. Afterwards there was no net-uptake of cations. With the high nitrogen treatment cations are taken up during the whole growing period studied. The straight broken line and the solid curve show that there is a sharp change in uptake rate at about 95 days. This change coincides with a change in the weather conditions during July and August 1969. The period between 52 and 95 days was rather sunny and dry and therefore several sprinkler irrigations were applied. Afterwards a period of cloudy weather followed. The average global radiation at Wageningen and data on rainfall and sprinkler irrigation are given in Table 9. A higher transpiration of the plants between 52 and 95 days than between 95 and 137 days is a tentative explanation of the change in cation uptake rate at 95 days. The change in weather conditions did not affect the dry matter production of the high nitrogen treatment, see Fig. 6.

In Fig. 8 the total cation content of the dry matter (ΣC) is given. Obviously the cations were diluted by the dry matter produced, as the content rapidly declines with time. Therefore, in Chapter 5 redistribution is studied by a reference method in stead of comparing contents of a plant part at different intervals of the growing period. The interruption of the trend in both treatments at about 95 days may be explained by the change in cation uptake rate.

The amount of inorganic anions taken up by the plants during the growing period is given in Fig. 9. Up to 109 days the amount taken up increased regularly. Thereafter there was no net-uptake of anions for the low nitrogen treat-

Table 9. Meteorological data of the growing period.

Days after germination	Average global radiation at Wageningen in cal per cm^2 per day*	Rainfall** in mm	Sprinkler irrigation in mm
52 - 66	382	39.5	30
66 - 81	421	6	65
81 - 95	488	54	30
95 - 109	242	71	-
109 - 137	284	7	-

* Calculated from data provided by the Laboratory of Physics and Meteorology.

** Data recorded at the Wageningen-Hoog trial fields of the Department of Field Crops and Grassland Husbandry.

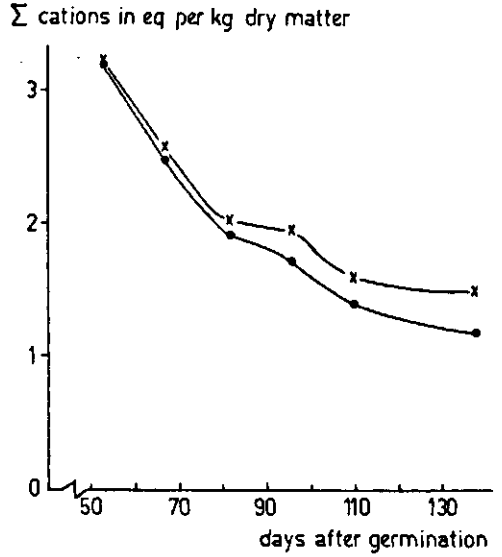


Figure 8. Content of the sum of cations during the growing period. . = 60 kg N per ha and x = 250 kg N per ha.

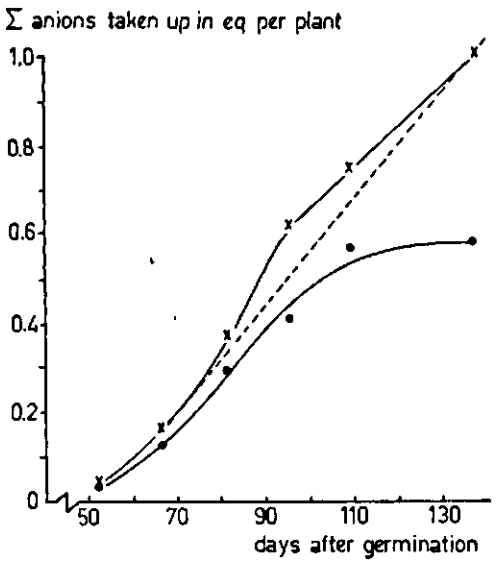


Figure 9. Amount of anions taken up by the plant during the growing period. . = 60 kg N per ha and x = 250 kg per ha.

ment, while with the other treatment the rate of uptake diminished after 95 days.

In Table 10 the data on ion uptake and utilization are given. From these data for sum of C and sum of A it is concluded:

- that the sugar-beet plants of both treatments take up inorganic cations and anions in equivalent amounts, the somewhat differing values in the last har-

Table 10. Ion uptake and utilization (per kg dry matter) during the growing period at two N applications.

Days after germination	60 kg N per ha					250 kg N per ha				
	ΣC in meq	ΣA in meq	Org-N in mmole	(C-A) in meq	sum of carboxylates in meq	ΣC in meq	ΣA in meq	Org-N in mmole	(C-A) in meq	sum of carboxylates in meq
52	29.7	29.6	23.5	23.5	23.4	41.0	40.0	30.6	31.6	33.9
66	128.3	120.4	97.0	102.7	101.9	155.8	162.9	125.3	118.3	126.5
81	299.7	295.7	217.6	221.6	244.2	354.5	374.0	269.9	250.7	269.5
95	436.5	410.0	315.7	334.3	336.7	613.4	623.4	491.9	482.5	482.5
109	549.2	572.0	439.8	412.6	439.1	701.8	750.9	567.7	518.3	593.1
137	518.9	587.9	460.6	392.8	382.9	977.4	1012.8	776.7	744.9	815.4

vests being due to loss of leaf material rich in inorganic cations and poor in inorganic anions and organic N;

- the uptake is markedly increased after dressing with nitrate nitrogen.

The ratio between the sum of inorganic anions taken up by the plant and the dry matter weight of the plant equalled at every harvest the sum of cation content as given in Fig. 8. However, the real sum of inorganic anion content in the dry matter was always much lower because of nitrate and sulphate reduction in the plant.

The amount of organic N per plant is given in Fig. 10. As seen from the sum of A and organic-N data in Table 10, about 70 - 80% of the anions taken up were transformed into organic N. The course of the curves in Fig. 10 shows that the organic-N production closely followed the ion uptake and that this production was increased by nitrate dressings. Moreover, Fig. 10 shows that before 95 days not only ion uptake but nitrate reduction too was very much stimulated by the weather conditions.

In Fig. 11 the amount of (C-A) as well as the sum of carboxylates per plant are given. Obviously, the positive (C-A) value is neutralized by negatively charged carboxylates, as (C-A) = sum of carboxylates. The data given in Table 10 for organic N, (C-A) and sum of carboxylates show that in addition the equality: (C-A) in eq ≈ organic N in mole holds. It has been shown that nitrate reduction determines the organic-N as well as the carboxylate production, when nitrogen is given as nitrate (Houba et al., 1971; Houba, 1973).

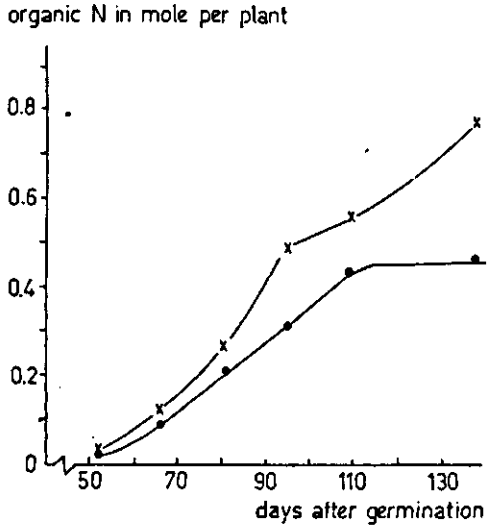


Figure 10. Amount of organic N per plant during the growing period.
. = 60 kg N per ha and
x = 250 kg N per ha.

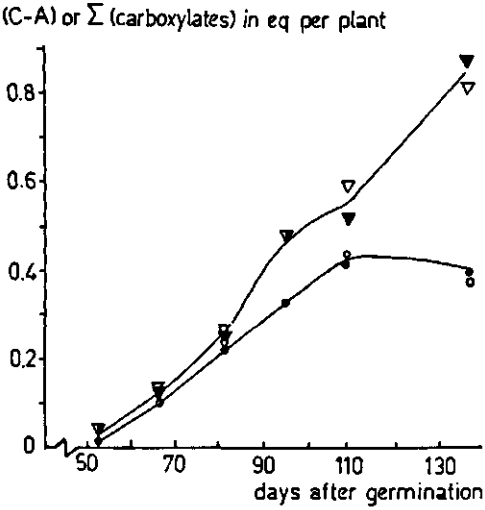


Figure 11. Amount of carboxylates per plant during the growing period.
Sum of carboxylates o = 60 kg N per ha, ▽ = 250 kg N per ha.
(C-A) . = 60 kg N per ha,
▼= 250 kg N per ha.

Moreover, it was concluded in these publications that with a nitrogen shortage organic-N production stops but that of carboxylates goes on, due to the alkaline uptake. However, in the present low nitrogen treatment, the nitrate stock was depleted at about 109 days after germination. Even after this date (C-A) equalled organic N, see Table 10. An explanation for this difference in experimental results may be sought in the moment the nitrate stock was depleted. In the experiments of Houba this was at about 60 days after germination and for the present low nitrogen treatment at about 109 or about 50 days later.

The course of the curves in Fig. 11 reveals that the carboxylate production closely follows ion uptake and utilization (Figs. 7, 9 and 10). It is stressed again that the sugar-beet plant fed with nitrate replaces most of the inorganic salts by organic salts.

The foregoing confirms a generally reported conclusion (Hoagland & Broyer, 1936; Ulrich, 1941; Chouteau, 1960; Coïc et al., 1961; de Wit et al., 1963; Dijkshoorn, 1964; van Tuil, 1965): large changes in pH of the cell solution due to ion uptake and utilization are prevented by changes in the carboxylate pool, besides the accumulation of carboxylates increases the buffer capacity (Small, 1946).

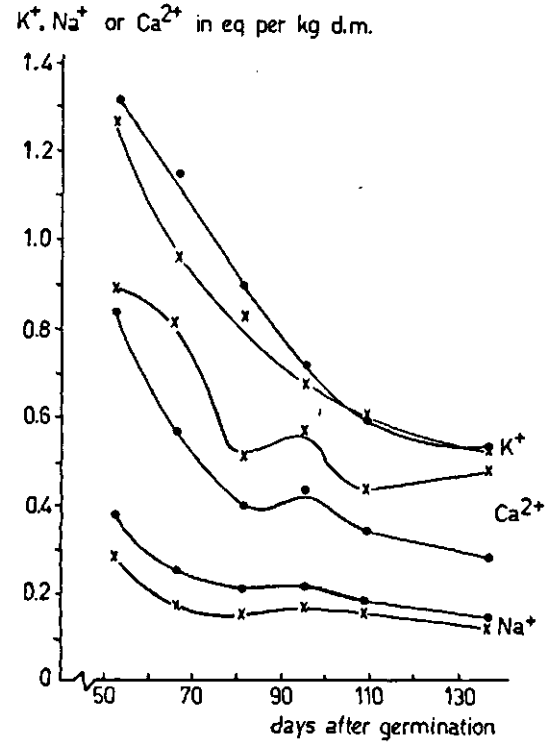
The normal (C-A) concept (de Wit et al., 1963; van Tuil, 1965) is confirmed in that the high yielding treatment has a higher (C-A) than the low yielding one, about 1130 and 900 meq (C-A) per kg dry matter at 137 days, respectively. However, during the growing period the (C-A) content decreases by about a factor 4 (Table 3, Fig. 6 and Table 10). The question - which (C-A) content is the "normal" one - remains. In fact in this study the relation between (C-A) and yield is a pseudo relation, derived from the organic-N and dry matter production. That in some plant species the (C-A) content is of the same magnitude as the organic-N content has already been reported by de Wit et al. (1963).

The content of the various inorganic cations is given in Figs. 12a and 12b. Like the sum of cation content, see Fig. 8, the contents diminish in both treatments with about a factor 2 between 52 and 137 days, because of dilution by the dry matter produced.

The Na content and to a lesser degree the K content is lowest, while the Ca and the Mg contents are highest with the high nitrogen treatment, because of the extra amount of nitrate of lime.

As in Fig. 8 there is an interruption of the general trend at about 95 days in the curves for Ca, Mg and Na but not for K. It is generally accepted (Barber, 1962) that supply to the roots by mass flow is important for Ca and Mg and by diffusion is important for K and H_2PO_4. The cation uptake rate changed at 95 days and this is a result of a change in transpiration rate. These results indicate the importance of Ca and Mg supply by mass flow to the roots of sugar-beet.

The content of the various inorganic anions is given in Figs. 12b and 12 c. Only the NO_3 content is of the same order of magnitude as the cation contents. From the results presented in Table 10 and Fig. 12b it can be calculated that about 90% of the inorganic anions taken up were nitrate ions. In contrast to the uniform decrease in the cation contents, the contents of the various inor-



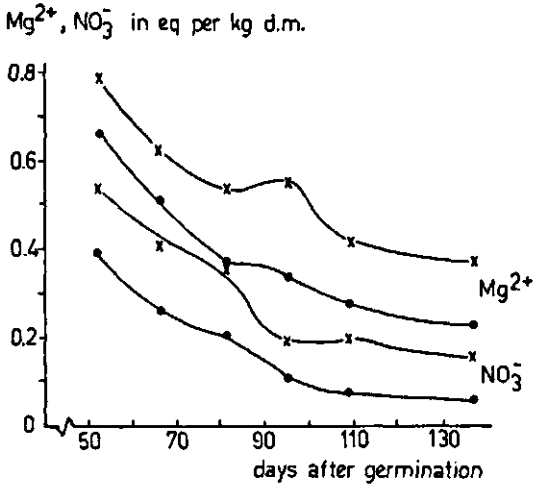
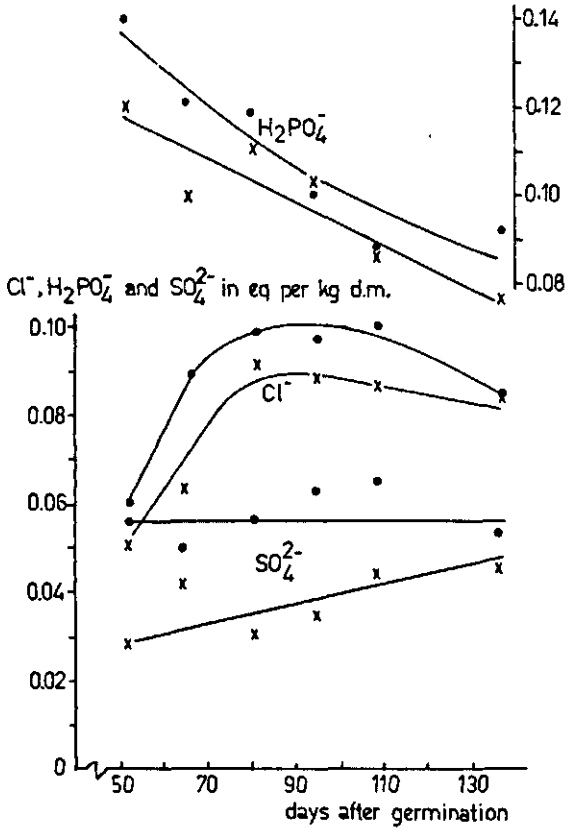


Figure 12. (a, b and c)
Contents of the various inorganic ions in the plant during the growing period.
. = 60 kg N per ha and
x = 250 kg N per ha.

ganic anions increased, decreased or hardly changed during the growing period.

Due to dilution by the dry matter produced and due to reduction the nitrate content decreases sharply during the growing period. In the low nitrogen treatment the content is after 95 days less than 100 meq NO_3 per kg dry matter, which



means nitrogen shortage.

The low nitrate content in the material harvested at 95 days supports the conclusion stated before that nitrate reduction was very much stimulated by the weather conditions before that date.

The chloride content increased up to about 95 days whereafter it did not change much. Possibly this result has also to be explained by the weather conditions favouring the transpiration in the period before 95 days.

The phosphate content decreased almost linearly with time, the relative decrease by a factor 1.5, however, being less than the relative decrease in the cations contents.

The sulphate content fluctuated, for the low nitrogen treatment between 50 and 65, for the high nitrogen treatment between 25 and 45 meq per kg dry matter. An organic-S content equivalent to 60 - 150 meq SO_4^{2-} per kg dry matter was calculated according to Dijkshoorn & van Wijk (1967). The fluctuations of the sulphate content are due to changes in the rate of uptake and

utilization of sulphate. The analytical method used for determining sulphate may contribute considerably to the fluctuations of the results. Therefore nothing can be concluded about changes in the sulphate content during the growing period.

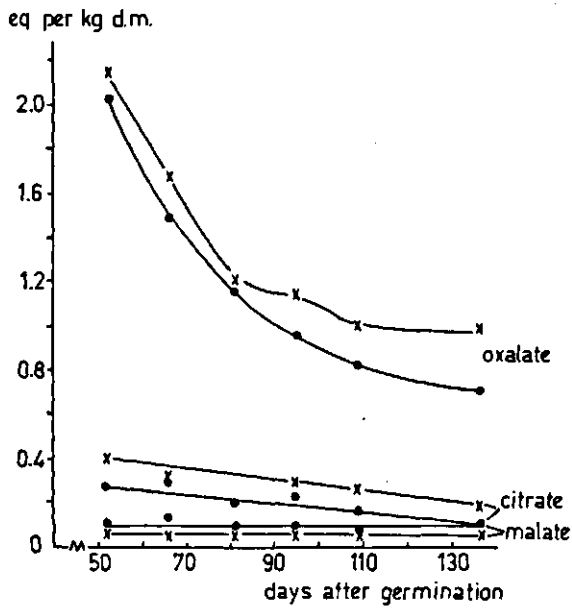


Figure 13. Contents of oxalate, malate and citrate in the plant during the growing period.
. = 60 kg N per ha and
x = 250 kg N per ha.

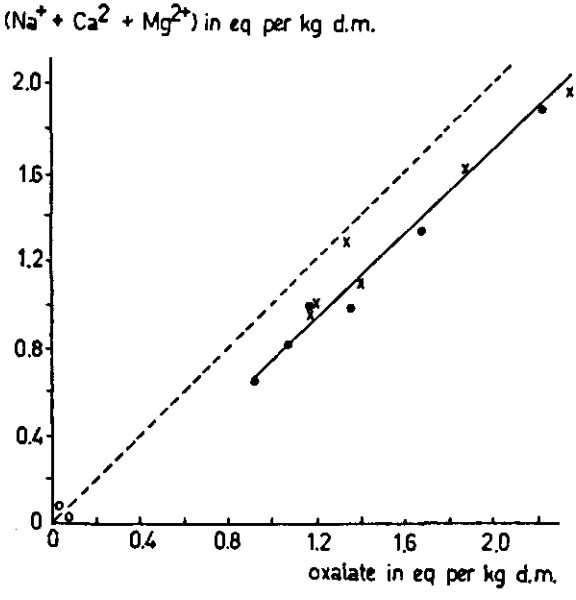


Figure 14. Relation between the sum of Na + Ca + Mg content and oxalate content in the sugar-beet plant.
. = 60 kg N per ha and
x = 250 kg N per ha.

The contents of the carboxylates present in quantitative important amounts are given in Fig. 13. Obviously the oxalate ion is the predominant anion in sugar-beet plants (compare Fig. 13 with Fig. 12).

Like the sum of cation content, the oxalate content diminishes with a factor 2 between 52 and 137 days.

The oxalate contents were highest for the high nitrogen treatment. This result agrees well with the higher contents of cations, organic N and (C-A)

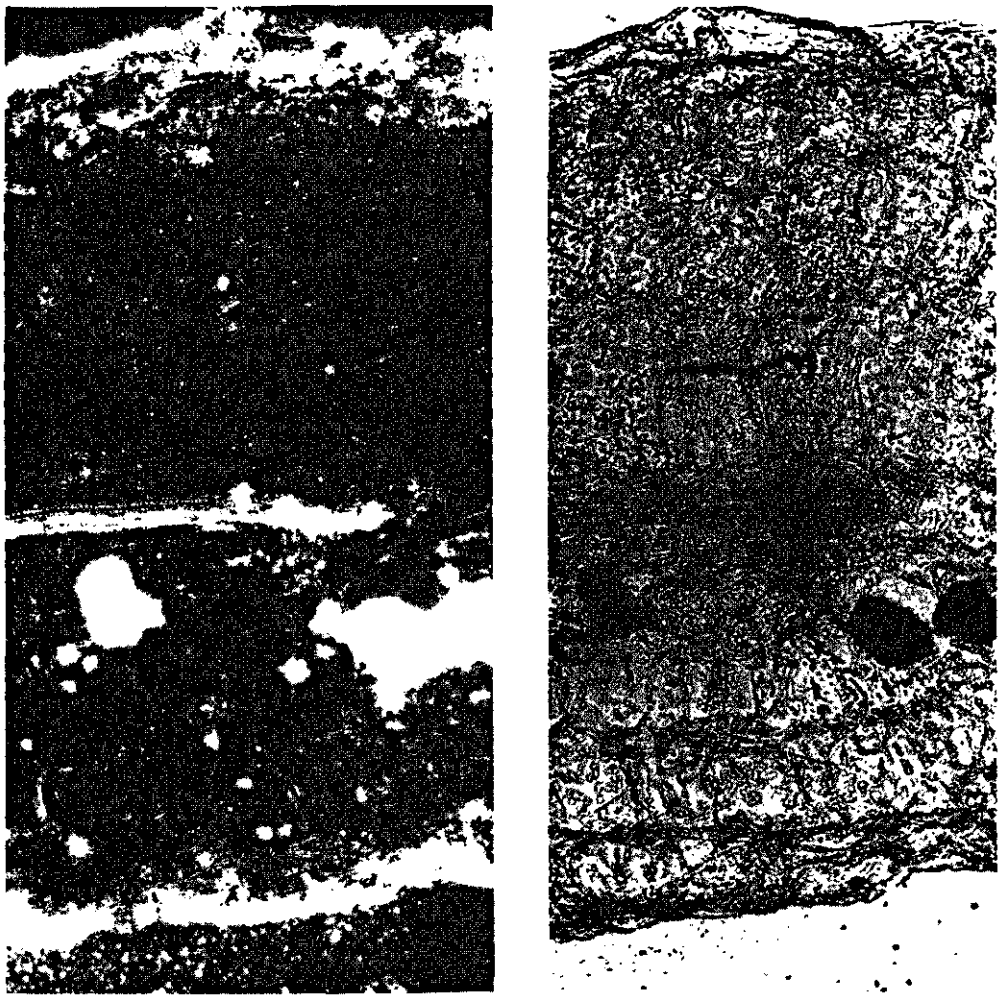


Figure 15. Beta leaf transverse, cut in H_2O, enclosed in Kaiser's gelatin-glycerin. The upper epidermis is at the top of the photograph,
a) photographed with polarized light,
b) photographed with U.V. light,
} by Dr Ir R.W. den Outer.

recorded for this treatment. The high oxalate content at 95 days indicates that the stimulated nitrate reduction favoured the oxalate production.

Citrate and malate contents were low and decreased during the growing period. However, they decreased relatively less than the oxalate content.

In Fig. 14 the sum of Na + Ca + Mg and the oxalate contents are given. From this figure it is concluded that during the growing period these cations and oxalate accumulated in equivalent amounts in the plants of both treatments, as previously described by van Egmond (1971) for leaf blade and petiole samples. However, a connection found between a cation or cations and oxalate does not necessarily mean, that the accumulation of the cations is caused by oxalate.

The correlation coefficient between the oxalate contents and the sum of Na + Ca + Mg or the sum of Ca + Mg contents is, in both treatments, equal $r_{(Na+Ca+Mg)-(oxalate)} = 0.990$ and 0.985, $r_{(Ca+Mg)-(oxalate)} = 0.991$ and 0.980, but there is only about 0.8 eq (Ca+Mg) on every equivalent oxalate. The stoichiometric relation only holds when Na too is taken into account.

The high oxalate content and the insolubility of certain oxalates suggest that oxalate crystals accumulate and this is confirmed by Fig. 15 where just beneath a tracheid large amounts of crystals are seen in the leaf. The end of the xylem in the leaves is mostly one or some tracheids thick. These tracheids are surrounded by parenchymatic cells. The crystals accumulate between the xylem-exit and the nitrate-reducing cells.

5 Distribution and redistribution of nutrients

5.1 INTRODUCTION

The carboxylates can be treated as a slag formed during nitrate reduction (Chouteau, 1960; van Egmond, 1971). However, this slag might influence the redistribution of the cations within the plant. For this reason the distribution and redistribution of inorganic ions as well as carboxylates and organic N are considered in this chapter.

The changes during the growing period in the distribution of C, A and organic N within the plant were analysed to evaluate which substances are redistributed and to what extent. The accumulation of ions in plant parts is the result of import and export processes. The organic anions are also affected by metabolic production and consumption within an organ, while N and S from NO_3 and SO_4 may be incorporated in organic molecules and exported in this form.

In general it is impossible to estimate quantitatively import, export, etc. by analysing time courses of accumulation only. Soil scientists take either titanium or silicon as an index element to calculate the amounts of other elements leached or accumulated from a part of the soil profile (C.E. Marshall). Similarly, the redistribution of elements in ionic or other forms within plants can be considered with respect to each other. Assuming that no or the same selective distribution takes place within the plant, we can calculate the amount of any element from its relation to the index element with

$$(A_r/E_r)U_i \qquad (1)$$

U_i is the amount of an element in the whole plant and A_r and U_r are the amounts of the index element in the organ and in the whole plant. Redistribution with respect to each other is then judged by comparing the calculated and actual amount in various organs.

Of course, the outcome of the procedure gains in value if an index element can be found that is not redistributed at all. Such an element may be silicon (Jones & Handreck, 1969) but its content was not determined because it does not contribute to the ionic balance. It would require also removing all adhering

soil from the fresh material which is practically impossible. N, S and P are unsuitable because they are - in organic molecules - redistributed throughout the plant. A cursory inspection of the data shows that the actual amount of K in older leaves decreased at the end of the growing period studied, so that this element was redistributed. One of the elements that continued to accumulate even in the older leaves was Cl and this was therefore chosen as the index element.

The difference between the calculated and the actual amount of an element gives the redistribution with respect to Cl. But it is mentioned now that during the subsequent analysis it will appear that Cl was not redistributed at all.

5.2 TIME COURSE OF NUTRIENT ACCUMULATION

The Cl content of leaf blades and petioles is plotted against leaf number for both N treatments in the Figs. 16 - 19. the Cl content on dry matter basis of the petioles is about 5 times as high as that of the leaf blades. The same is true for the NO_3 content. These high contents are partly due to the low dry matter content of the petioles. In the following sections leaf blade and petiole are treated as a whole.

The amount of Cl accumulated in the leaves, calculated for each sampling date from the dry weights and the contents of blades and petioles is given in Figs. 20 and 21. Random scattering was reduced by smoothing the data and curves were derived for the time course of Cl accumulation in Leaves 5, 10, 15, 20, 25, 30, 35 and 40. The data of the oldest leaves obtained at 109 days were treated with special care as loss or partial loss may have occurred. In Fig. 22 the time course of Cl accumulation in the root plus crown is given.

The amount of Cl in practically all plant parts increases monotonically with time in both treatments except for leaf number 5, which is due to leaf fall (Section 4.2) and except for the root plus crown of the high N treatment. Apart from these losses, there may have been some leaching. No direct information is available about leaching, but it is concluded from literature data (van Schreven, 1938; Tukey Jr., 1970) and the absence of long periods of rain that this phenomenon is not important.

Similar curves for the time course of accumulation can be computed for the other ions and for the nitrogen incorporated in organic molecules.

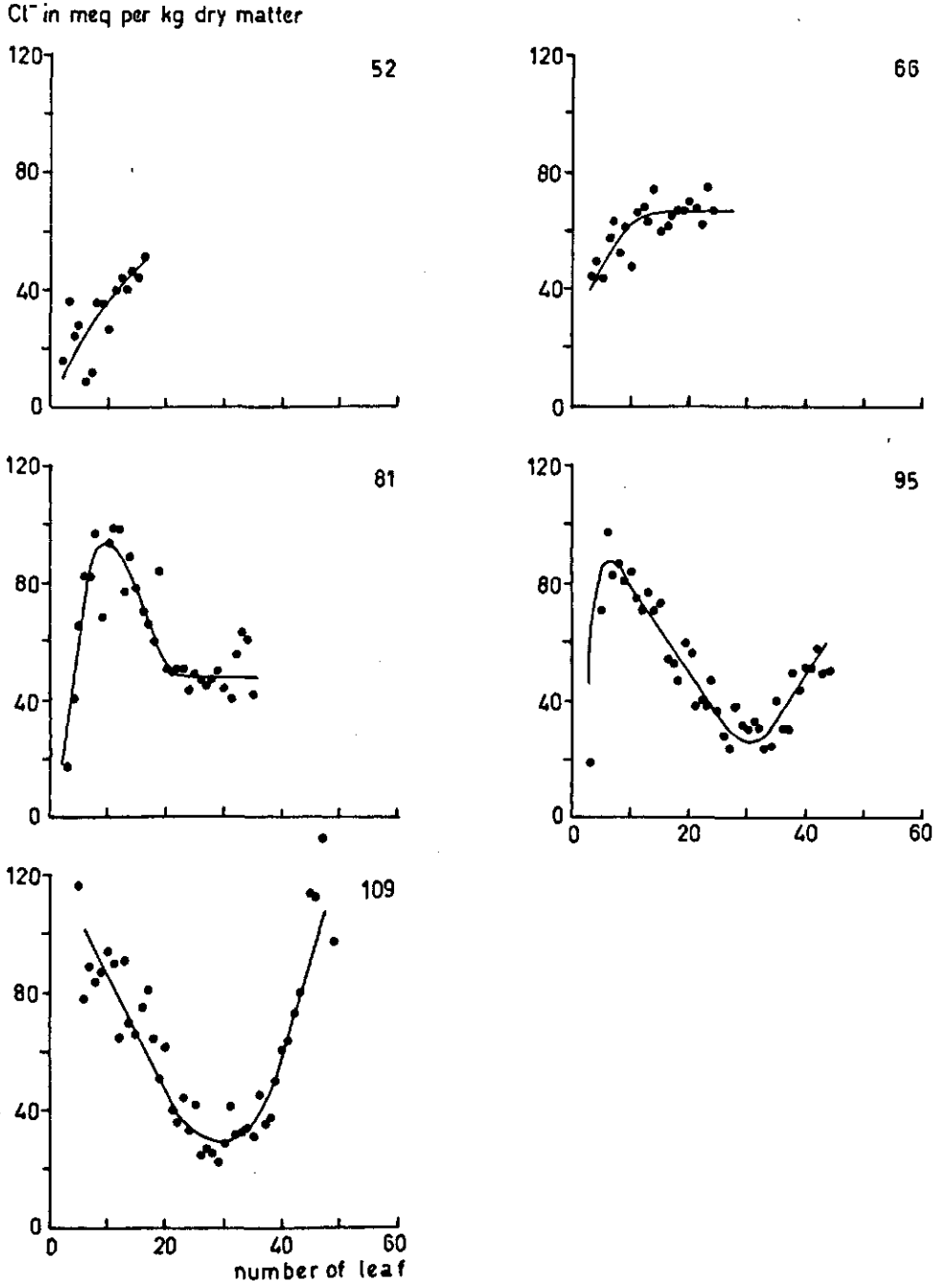


Figure 16. Cl content in the leaf blades at 52, 66, 81, 95 and 109 days after germination, 60 kg N per ha.

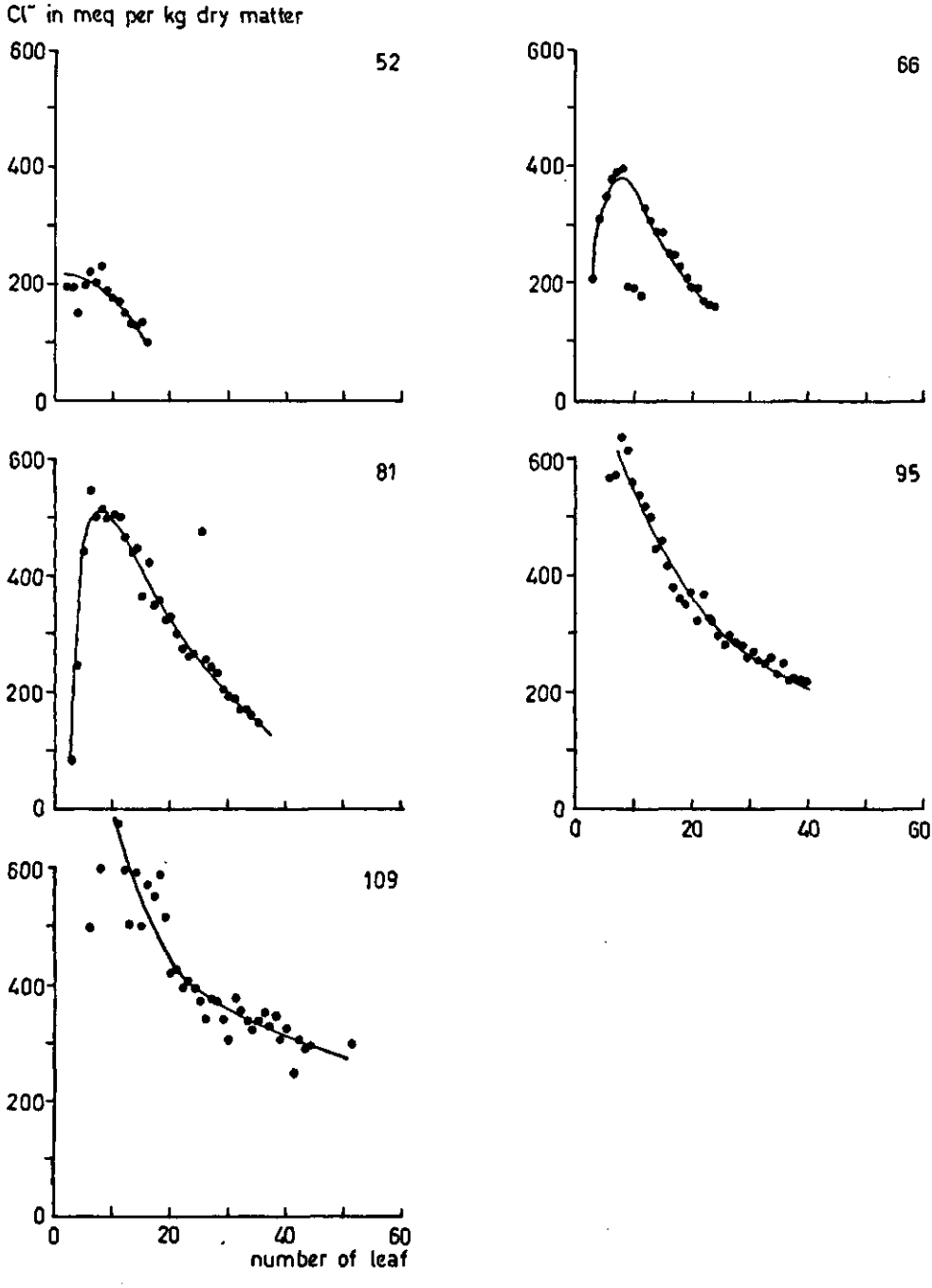


Figure 17. Cl content in the petioles at 52, 66, 81, 95 and 109 days after germination, 60 kg N per ha.

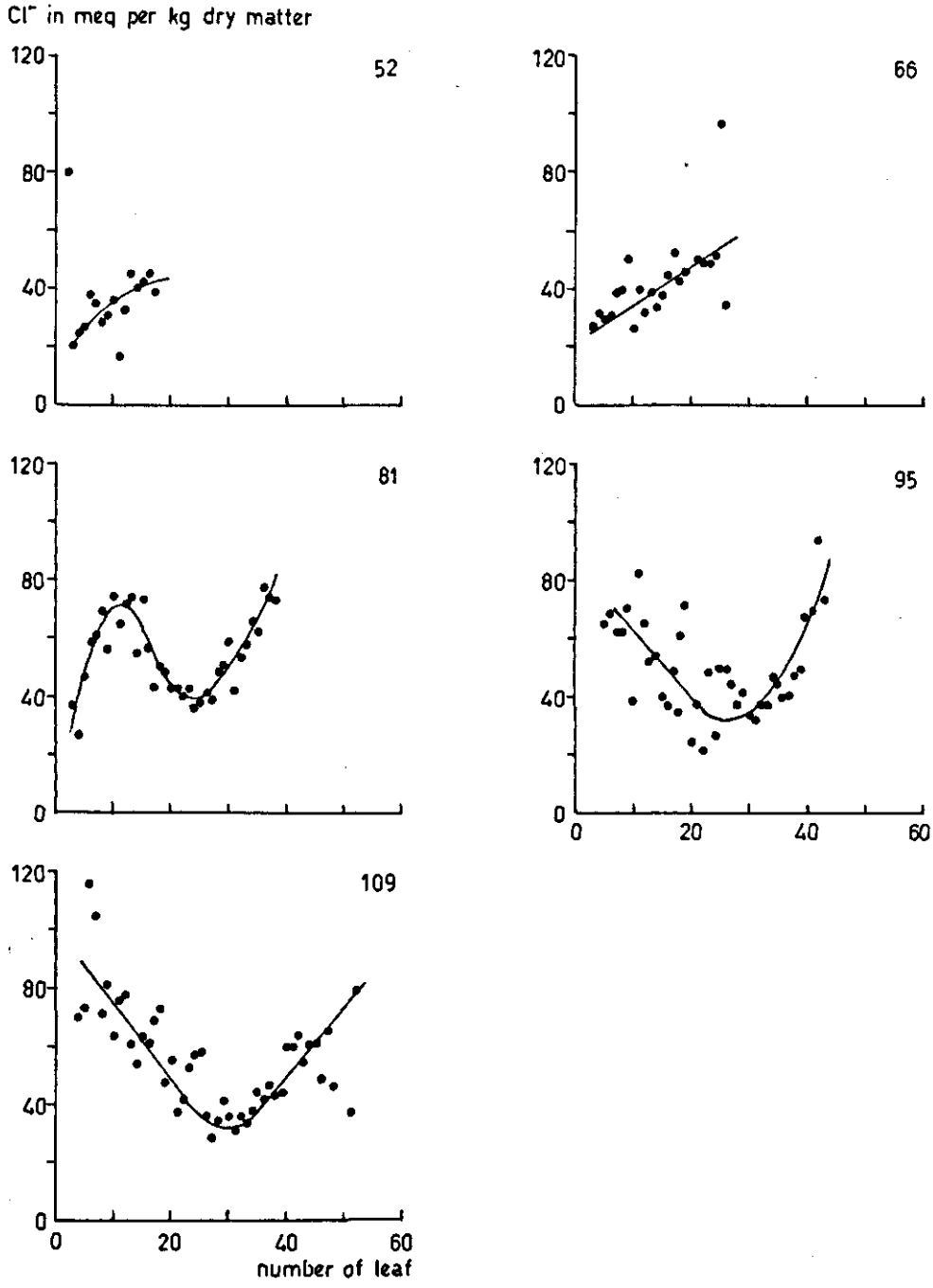


Figure 18. Cl content in the leaf blades at 52, 66, 81, 95 and 109 days after germination, 250 kg N per ha.

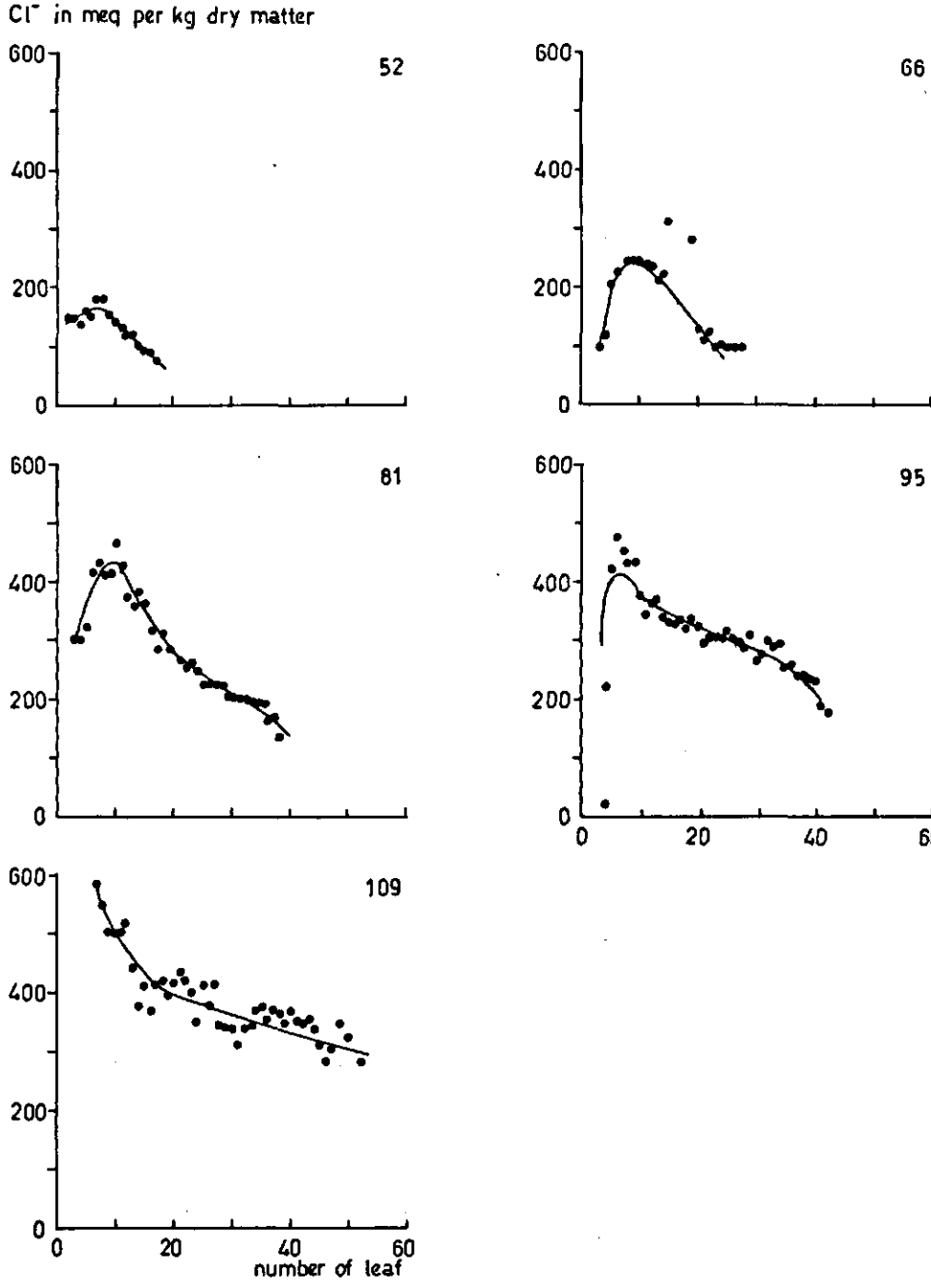


Figure 19. Cl content in the petioles at 52, 66, 81, 95 and 109 days after germination, 250 kg N per ha.

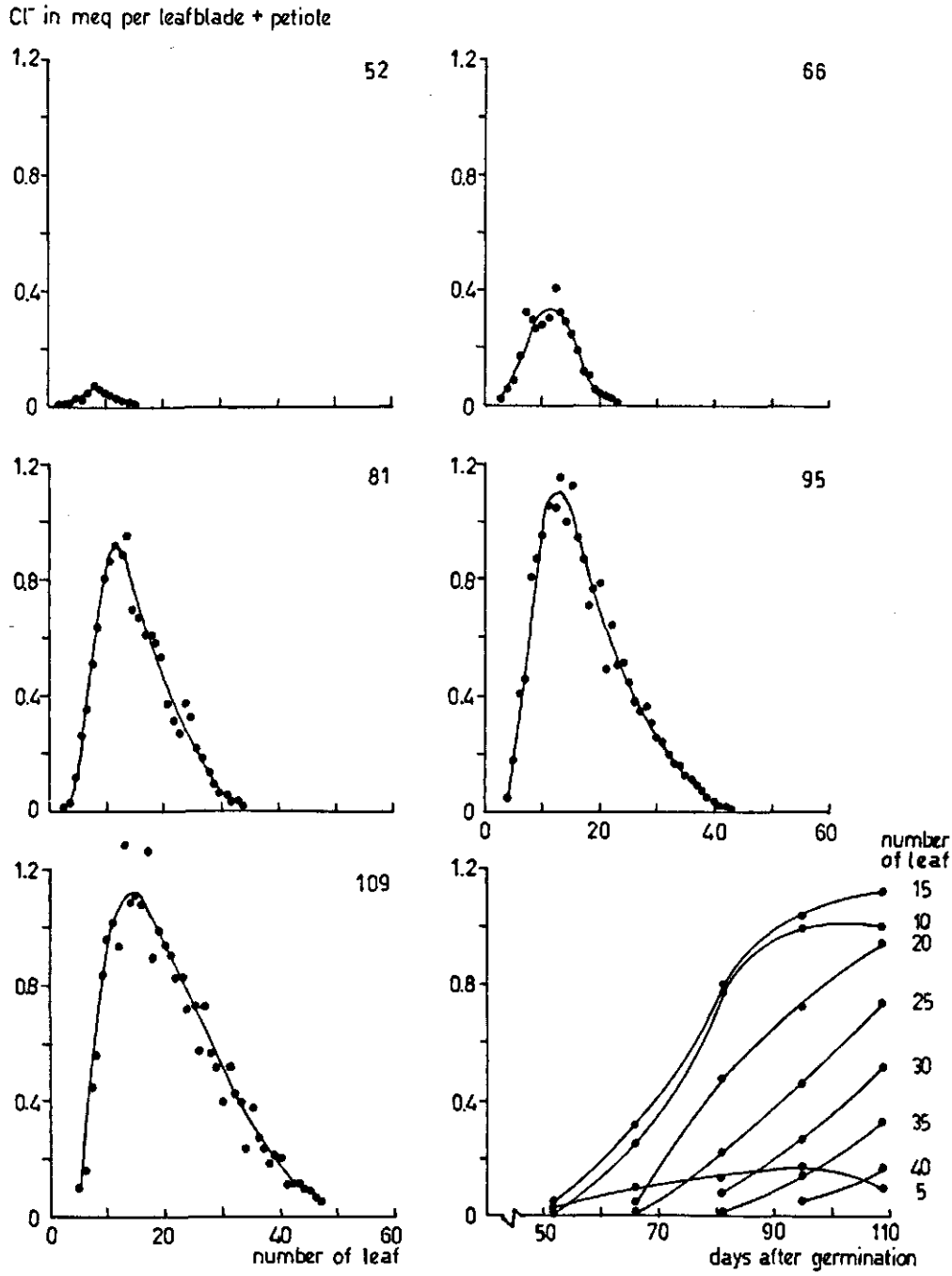


Figure 20. Amount of Cl per leaf during the growing period, 60 kg N per ha. The values plotted on the lower right graph are read from the lines in the other graphs.

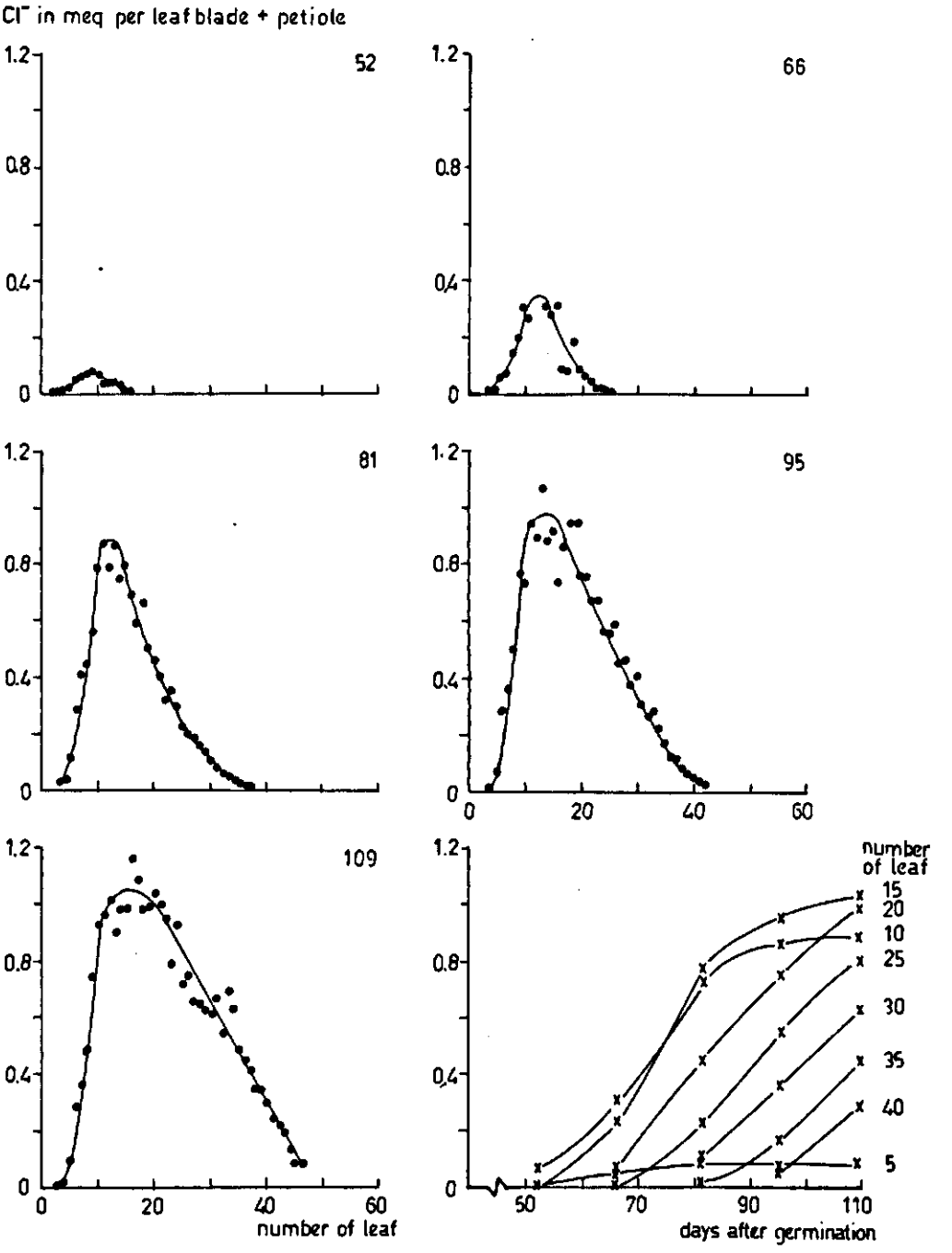


Figure 21. Amount of Cl per leaf during the growing period, 250 kg N per ha. The values plotted on the lower right graph are read from the lines in the other graphs.

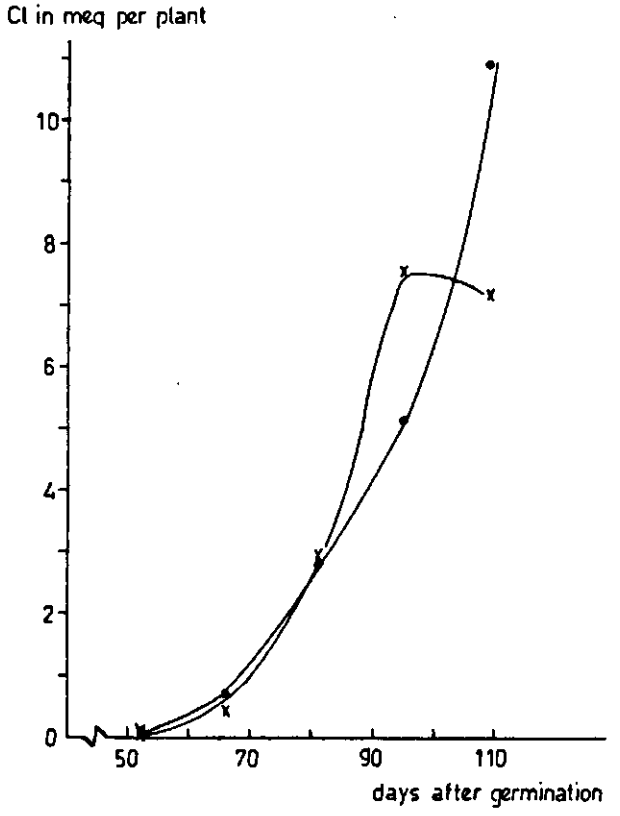


Figure 22. Amount of Cl in the sugar-beet and crown during the growing period.

5.3 REDISTRIBUTION OF NITRATE

The nitrate that moves into the organs does not remain there as such, but its nitrogen is incorporated in organic compounds, whereas the negative charge is found again in the organic anions. For the plant as a whole the organic-N content equals the (C-A) content (Section 4.3).

A comparison of the accumulated amount of NO_3 + (C-A) in the leaves and the roots and the calculated nitrate values, as done in Fig. 23 for the nitrogen treatments, shows that both values are practically the same. Hence, nitrate and the carboxylates that constitute the (C-A) package are redistributed in a similar way to Cl throughout the plant. This is obviously so when Cl and (C-A) both do not redistribute at all.

The carboxylates that are formed are mainly insoluble oxalates (Section 4.3) and it seems highly improbable that these and Cl and NO_3 are redistributed at the same relative rate. Therefore it is concluded that none of these ions are redistributed and that Cl is a good index to study the redistribution

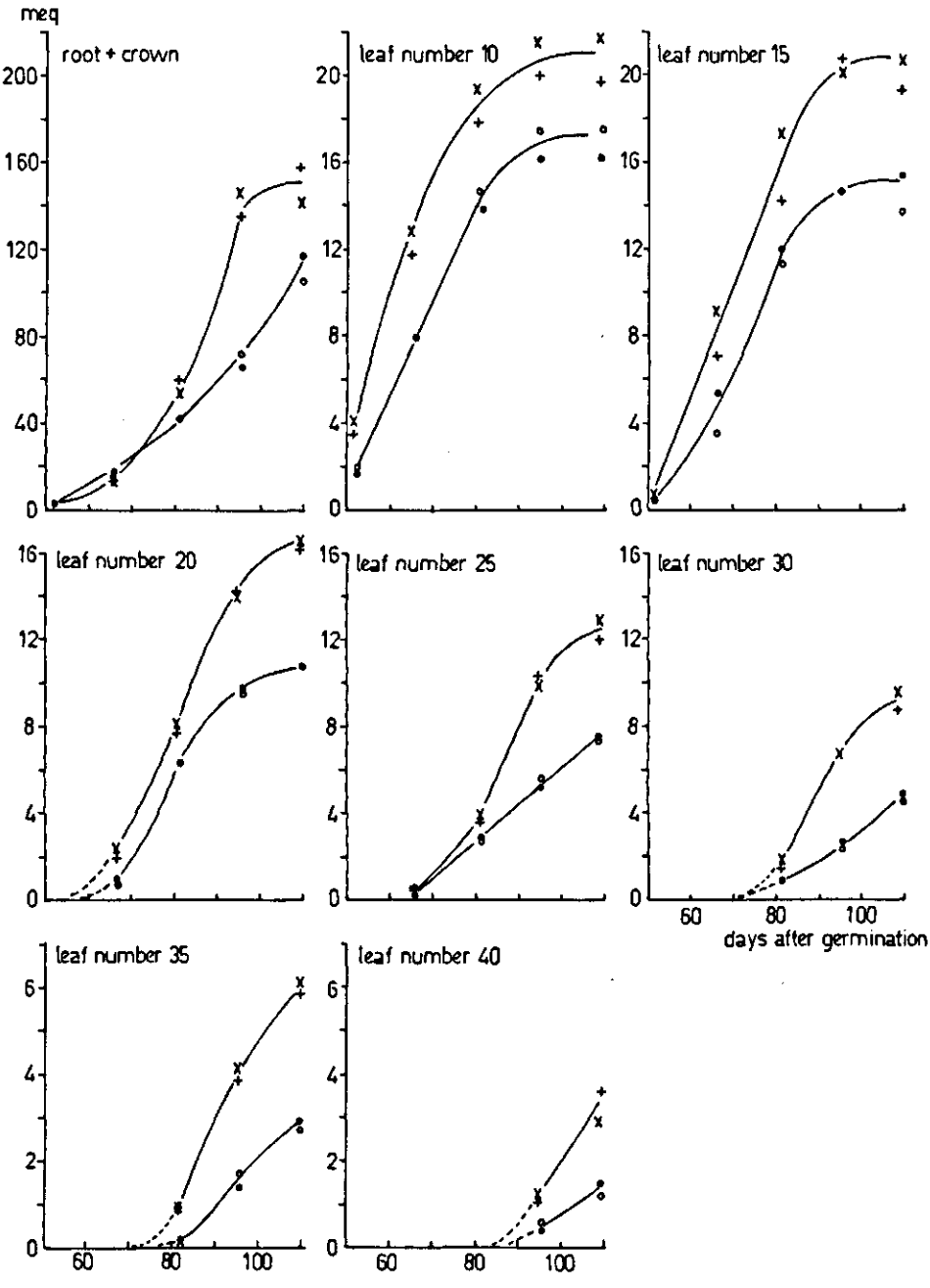


Figure 23. The calculated nitrate transport towards various plant parts.

. = with Cl as reference
o = NO_3 + (C-A)
} 60 kg N per ha

x = with Cl as reference
+ = NO_3 + (C-A)
} 250 kg N per ha.

of the elements. Corollaries of this conclusion are: that the slag of the organic-N production from NO_3 and organic compounds remains where it is formed, so that the (C-A) content of an organ is equivalent to the nitrate reduction that has taken place during the life time of this organ, as supposed by Chouteau (1960); and secondly, because crown and root have a certain low (C-A) content, that nitrate is reduced mainly in the leaves and to some extent in the crown plus root.

5.4 REDISTRIBUTION OF NITROGEN

The (C-A) and the organic-N amounts in various organs are given in Fig. 24 for both N treatments. At any moment and in any organ where the slope of the organic-N curve is steeper than the slope of the (C-A) curve net import of organic N occurs, while in the opposite case organic N is exported.

Inspection of the data shows that only a limited nitrate reduction took place in root plus crown and that during their life time, there is considerable import of organic N from the leaves. At the same time, there occurs considerable transfer of nitrogen between leaves so that:

- leaves with numbers up to about 20 exported a great deal of the organic N they have produced, e.g. leaf 10 about 70% at 109 days;
- leaves with numbers of about 25 have produced about the amount of organic N present at 109 days, so did the younger leaves of the high N treatment;
- leaves with numbers over 25 of the low N treatment have imported much of their organic N from the old leaves, e.g. leaf 40 about 30 - 50% at 109 days.

In Fig. 25 the redistributed amounts of organic N are given. The organic N was redistributed from the leaves towards the root plus crown and from old leaves towards young leaves. In root plus crown of both treatments about 50% of the organic N present was redistributed organic N (109 days).

The time when a young leaf becomes self-supporting for organic N is mainly determined by the organic-N import and the nitrate supply. At the end of the growing period, the growth of the young leaves in the low N treatment depends on the part of the redistributed organic N that reaches these leaves, while at the same time in the high N treatment the young leaves are supplied with nitrate and become self-supporting.

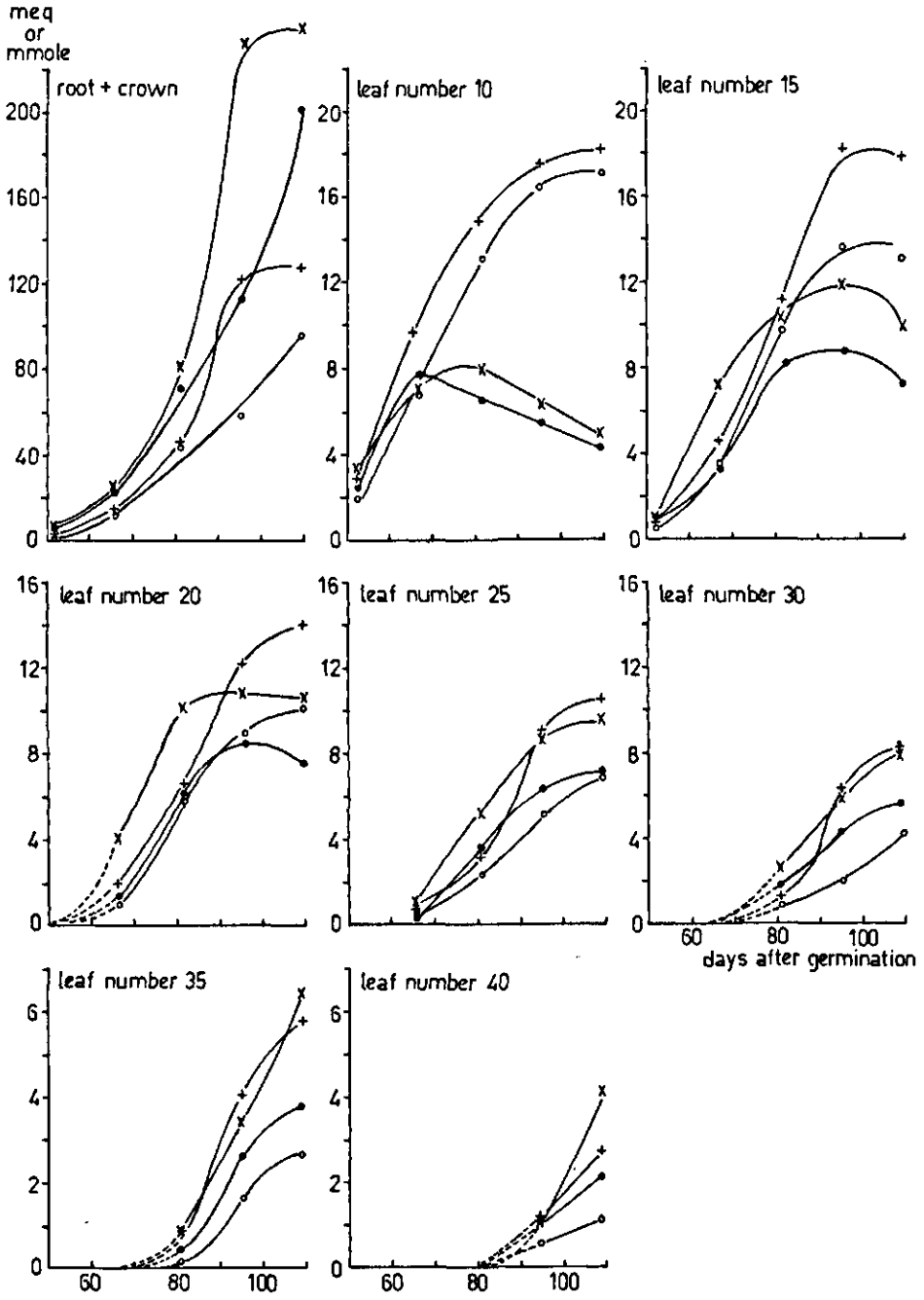


Figure 24. Amount of organic N and the amount (C-A) in various plant parts of the sugar-beet plant during the growing period.

. = organic N
o = (C-A)
} 60 kg N per ha

x = organic N
+ = (C-A)
} 250 kg N per ha

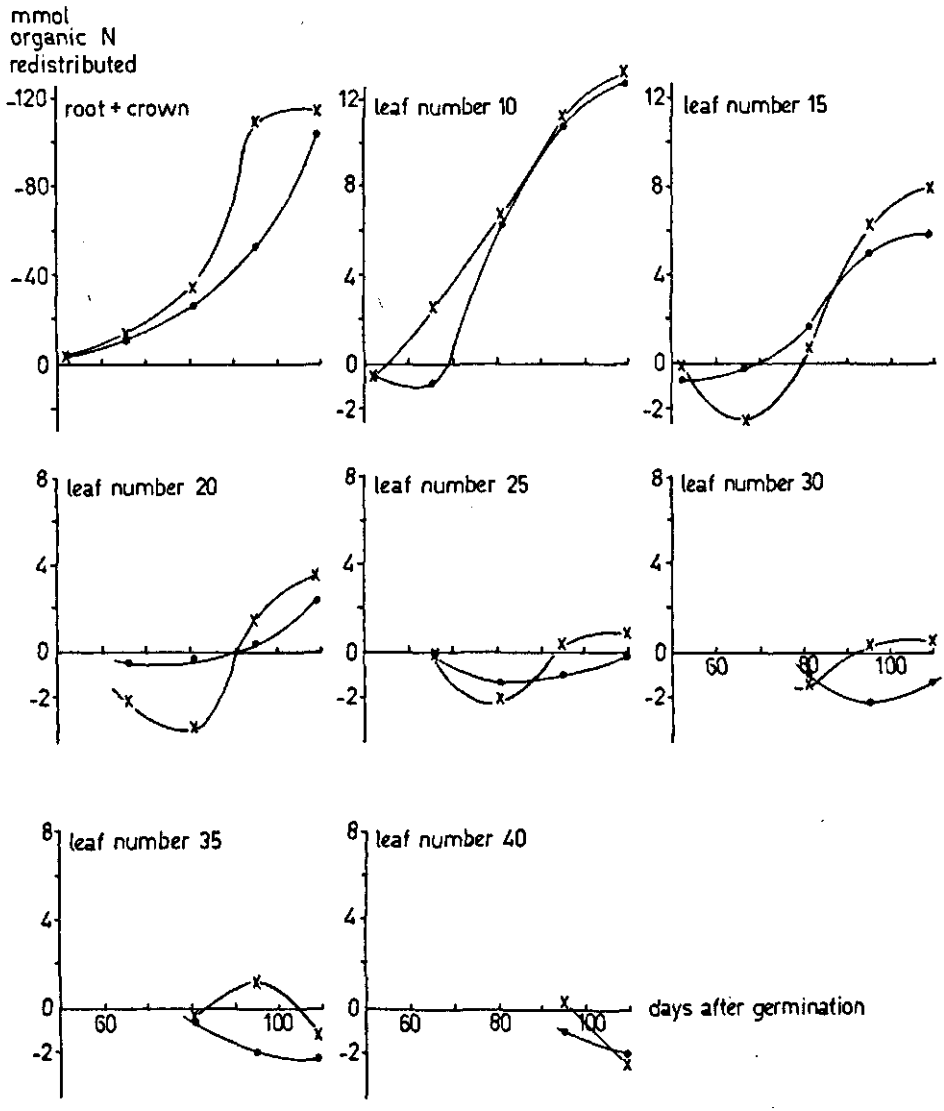


Figure 25. Amount of organic N redistributed.

. = 60 kg N per ha

x = 250 kg N per ha

5.5 REDISTRIBUTION OF INORGANIC CATIONS

The actual and the calculated sum of C values in various organs given in Fig. 26, are practically the same at any moment, in any organ and for both N treatments.

As 80 - 90% of the anions taken up is NO_3 and this anion is mainly transported to the leaves, accompanied by the bulk of the cations, and as the negative charge, imported with the NO_3 is stored in the accumulating carboxylates, the equality demonstrated in Fig. 26 is understandable.

The actual and the calculated Na amounts given in Fig. 27, show that Na is not redistributed within the plant, therefore Na would be as good an index as Cl in Eqn(1).

A comparison of the actual and the calculated K amounts, as made in Fig. 28, shows that these values differ markedly. Because of these differences it is concluded that:

- large amounts of K are redistributed towards root plus crown;
- large amounts of K are exported from the leaves when they grow old; even the actual K amount reveals export;
- some K is redistributed towards young leaves.

In root plus crown of the low and high N treatment about 35 and 50% respectively of the K present is redistributed K at 109 days.

Since the actual and calculated sum of C as well as Na amounts are equal, it follows that Ca or Mg are redistributed in quantitatively the same amounts as K, but in opposite direction. Since the Ca + Mg content, especially in the high N treatment, is much higher than the K content, the redistribution of divalent ions will be relatively less than that of K and will be less easily demonstrated by figures such as 28.

In Fig. 29 the actual and calculated Ca amounts appear to be different. It is concluded that:

- large amounts of Ca, up to 40%, are redistributed, especially in the high N treatment, from the root plus crown towards the leaves;
- large amounts of Ca are redistributed towards the leaves when they grow old;
- some Ca is exported from young leaves.

The actual and calculated Mg amounts, given in Fig. 30, differ especially for the high N treatment so that it is concluded that:

- Mg is redistributed to a small extent within the plants of the low N treatment;
- Mg is redistributed from the root plus crown towards the leaves in the high N treatment. At the end of the growing period about 20% is redistributed;
- Mg is redistributed towards the leaves when they grow old in the high N treatment;
- some Mg is exported from young leaves.

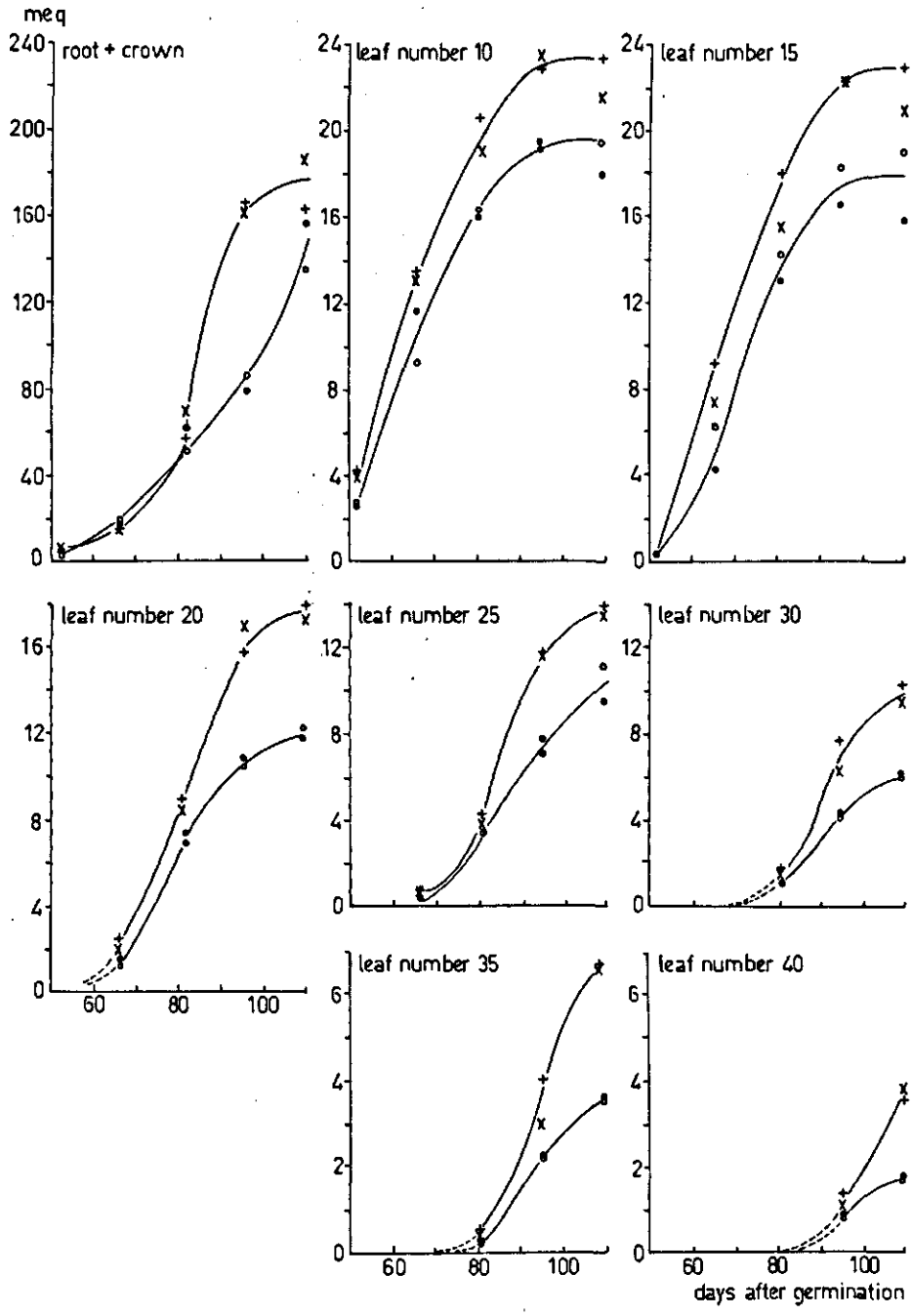


Figure 26. Sum of cations present in various plant parts and the sum of cations calculated with Cl as reference.

. = ΣC
o = ΣC calculated } 60 kg N per ha

x = ΣC
+ = ΣC calculated } 250 kg N per ha

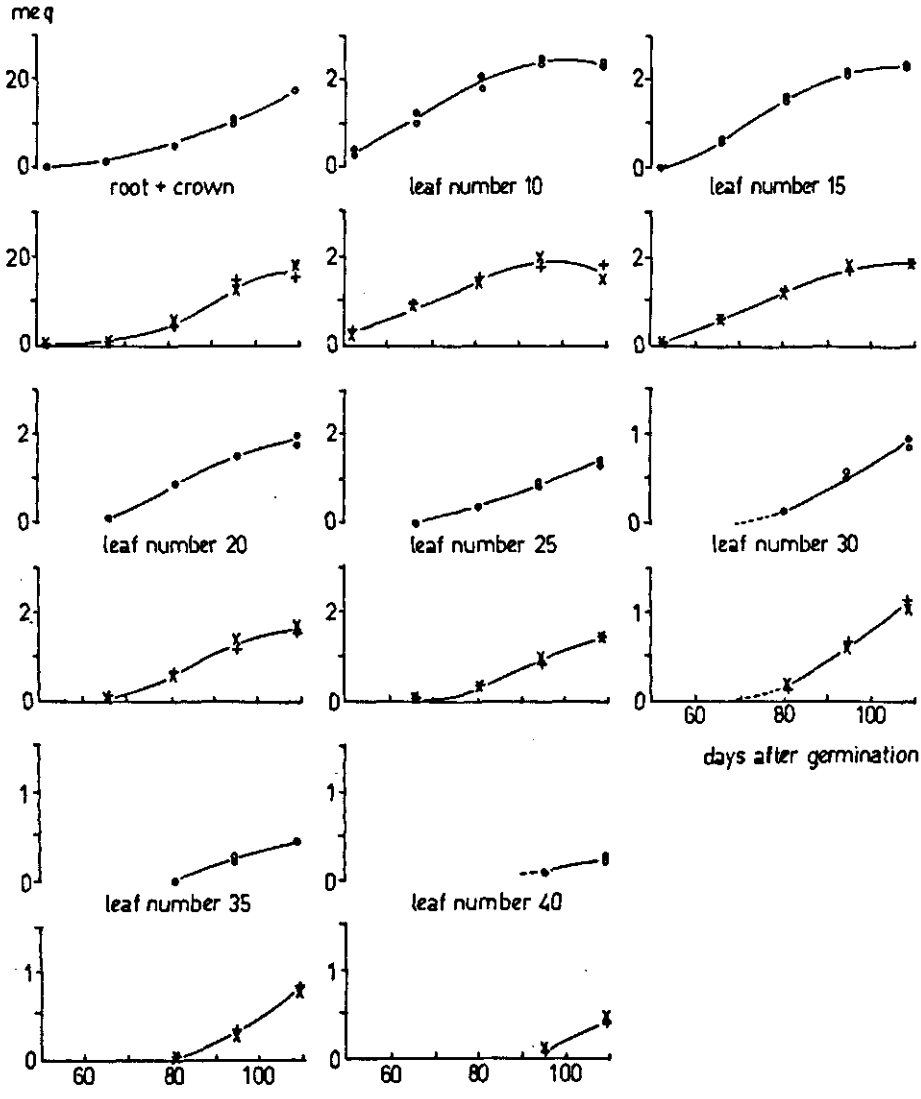


Figure 27. Amount of Na present in various plant parts and the amount of Na calculated with Cl as reference.

. = Na
o = Na calculated } 60 kg N per ha

x = Na
+ = Na calculated } 250 kg N per ha

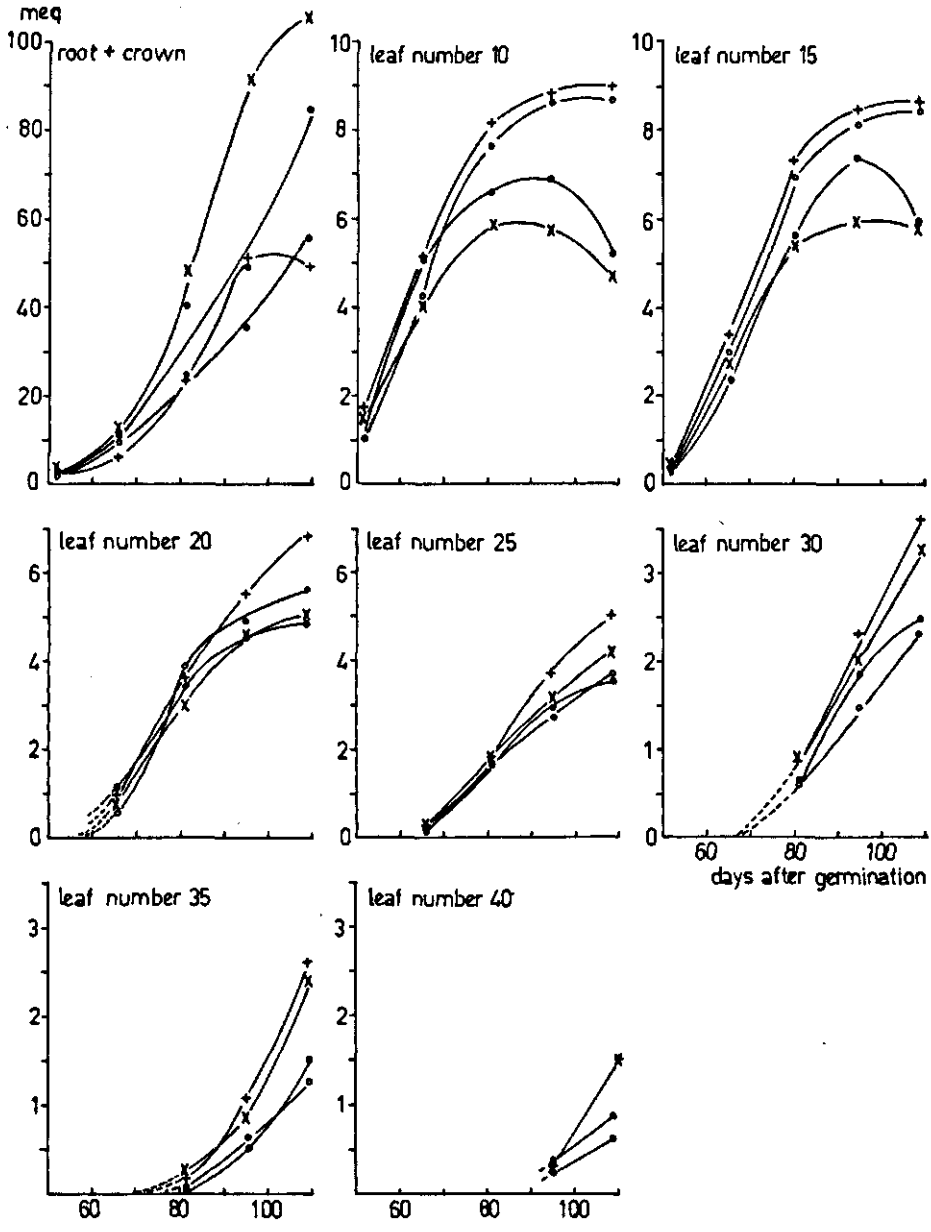


Figure 28. Amount of K present in various plant parts and the amount of K calculated with Cl as reference.

. = K
o = K calculated } 60 kg N per ha

x = K
+ = K calculated } 250 kg N per ha

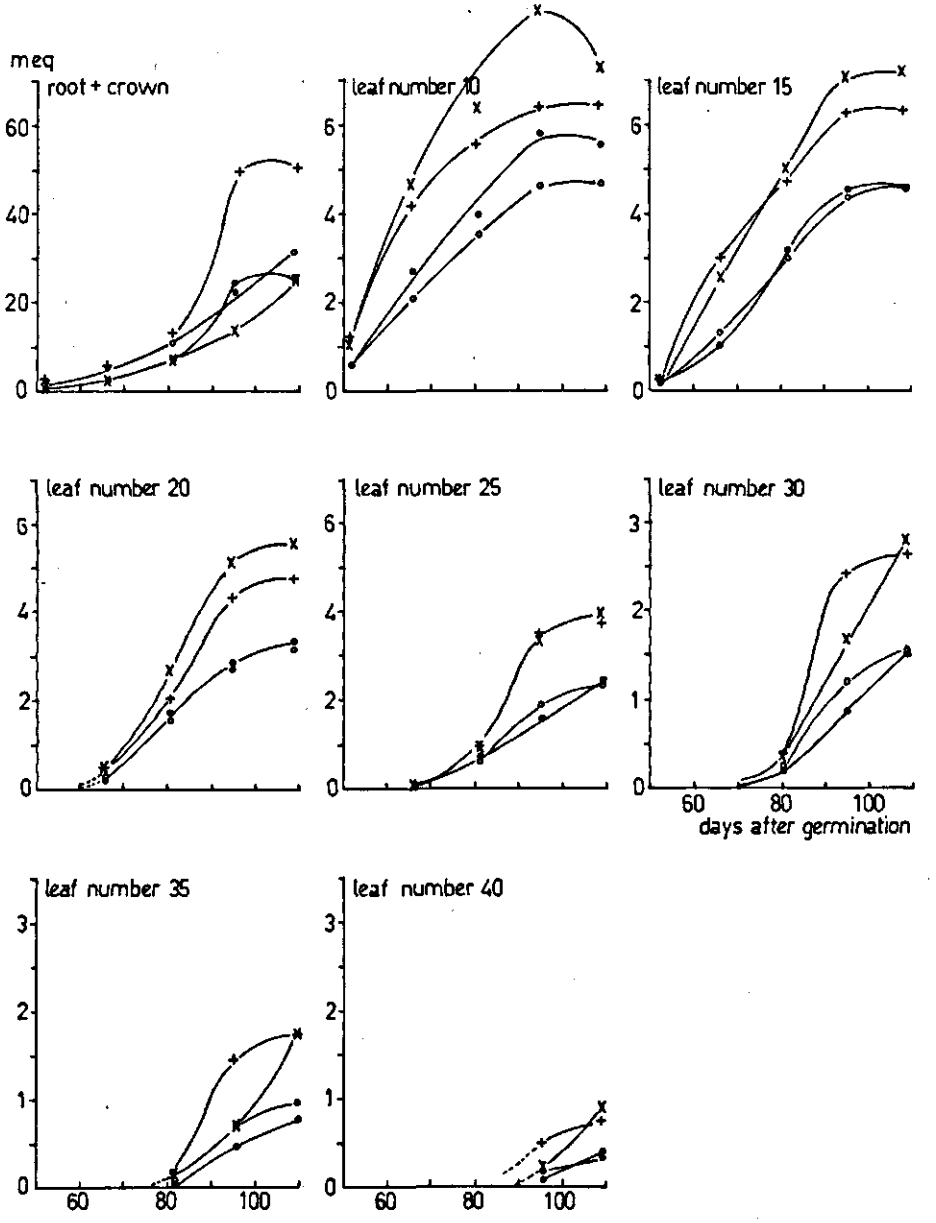


Figure 29. Amount of Ca present in various plant parts and the amount of Ca calculated with Cl as reference.

. = Ca
o = Ca calculated } 60 kg N per ha

x = Ca
+ = Ca calculated } 250 kg N per ha

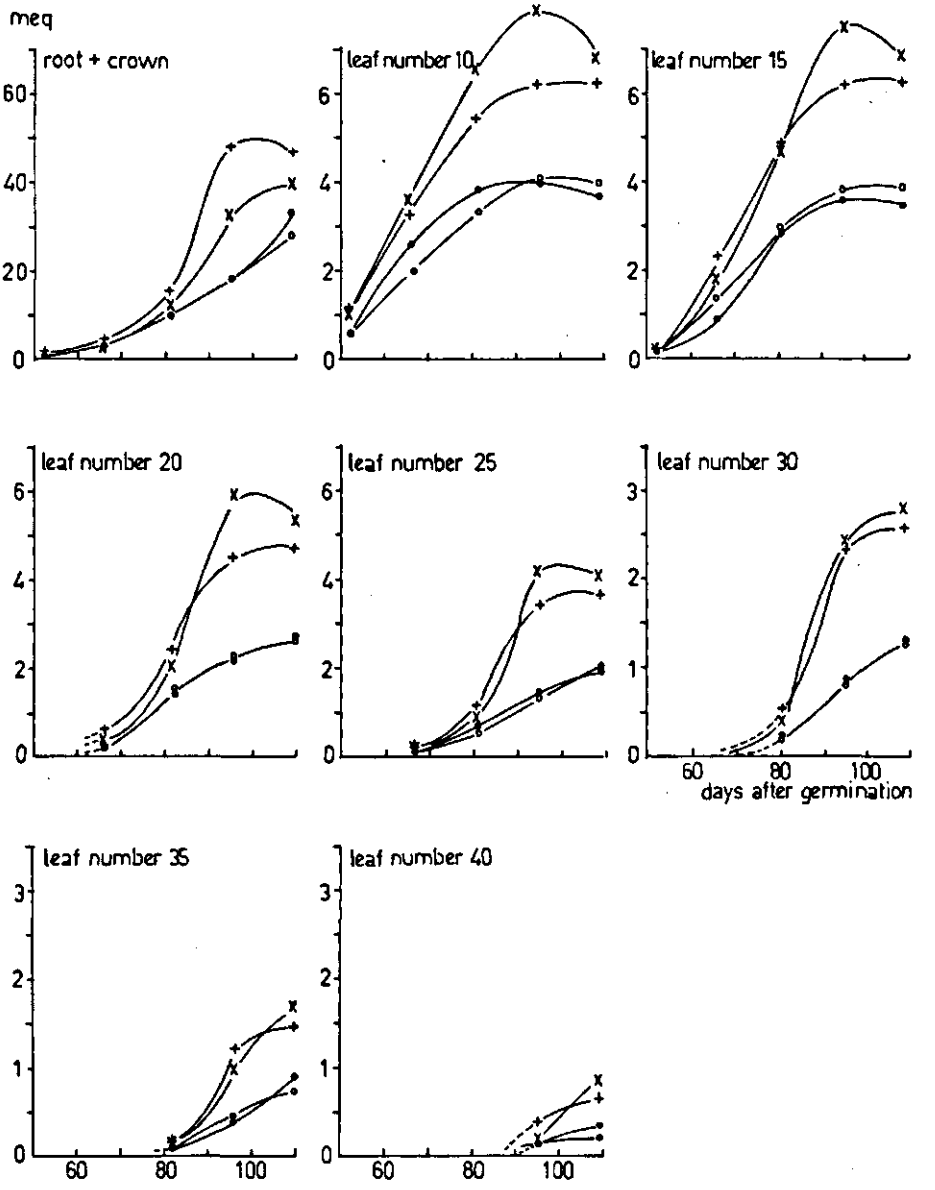


Figure 30. Amount of Mg present in various plant parts and the amount of Mg calculated with Cl as reference.

. = Mg
o = Mg calculated } 60 kg N per ha

x = Mg
+ = Mg calculated } 250 kg N per ha

The limited redistribution of the alkaline earths from the leaves agrees with the findings of Ringoet et al. (1968) and of Wiersum et al. (1971). Ringoet et al. (1968) assumed that the limited Ca redistribution is probably not due to the inability of Ca to move in the phloem but to a great accumulation and adsorption capacity of the various leaf tissues. The accumulation of carboxylates in especially the old leaves reported by van Egmond (1971) agrees well with this assumption.

5.6 REDISTRIBUTION OF INORGANIC ANIONS

Cl and NO_3 are considered in the sections 5.2 and 5.3. The SO_4 portion in the total inorganic anion uptake is small; moreover a part of this SO_4 is reduced and transferred into organic S. Data on the remaining SO_4 show that this anion accumulated monotonically in the various organs. From this it is concluded that SO_4 as such was not redistributed in quantitative important amounts.

In the plant, P is partially transformed from inorganic into organic phosphate. Of course the total P determination in the digests gives no information about the ratio inorganic to organic P. Therefore the amounts of P are given in equivalents H_2PO_4.

The actual and the calculated H_2PO_4 amounts, given in Fig. 31, differ markedly, so that

- large amounts of phosphate are redistributed towards root plus crown;
- large amounts of phosphate are exported from the leaves when they grow old;
- phosphate is redistributed towards young leaves.

In root plus crown of both treatments about 55% of the phosphate present is redistributed phosphate at 109 days.

It is stressed that the foregoing conclusions mean that inorganic anions are hardly redistributed.

5.7 THE CATION REDISTRIBUTION PROCESS

As the redistribution of K is counterbalanced by that of Ca and Mg, net anion redistribution may be very small, but nevertheless the redistributing cations must be accompanied by anions.

Carboxylates are present in phloem exudates and according to de Wit et al. (1963) and Dijkshoorn et al. (1968) potassium and carboxylates are transported through the phloem. Decarboxylation in the root of sugar-beet could also provide HCO_3^- for the Ca and Mg redistribution through the xylem, as may be

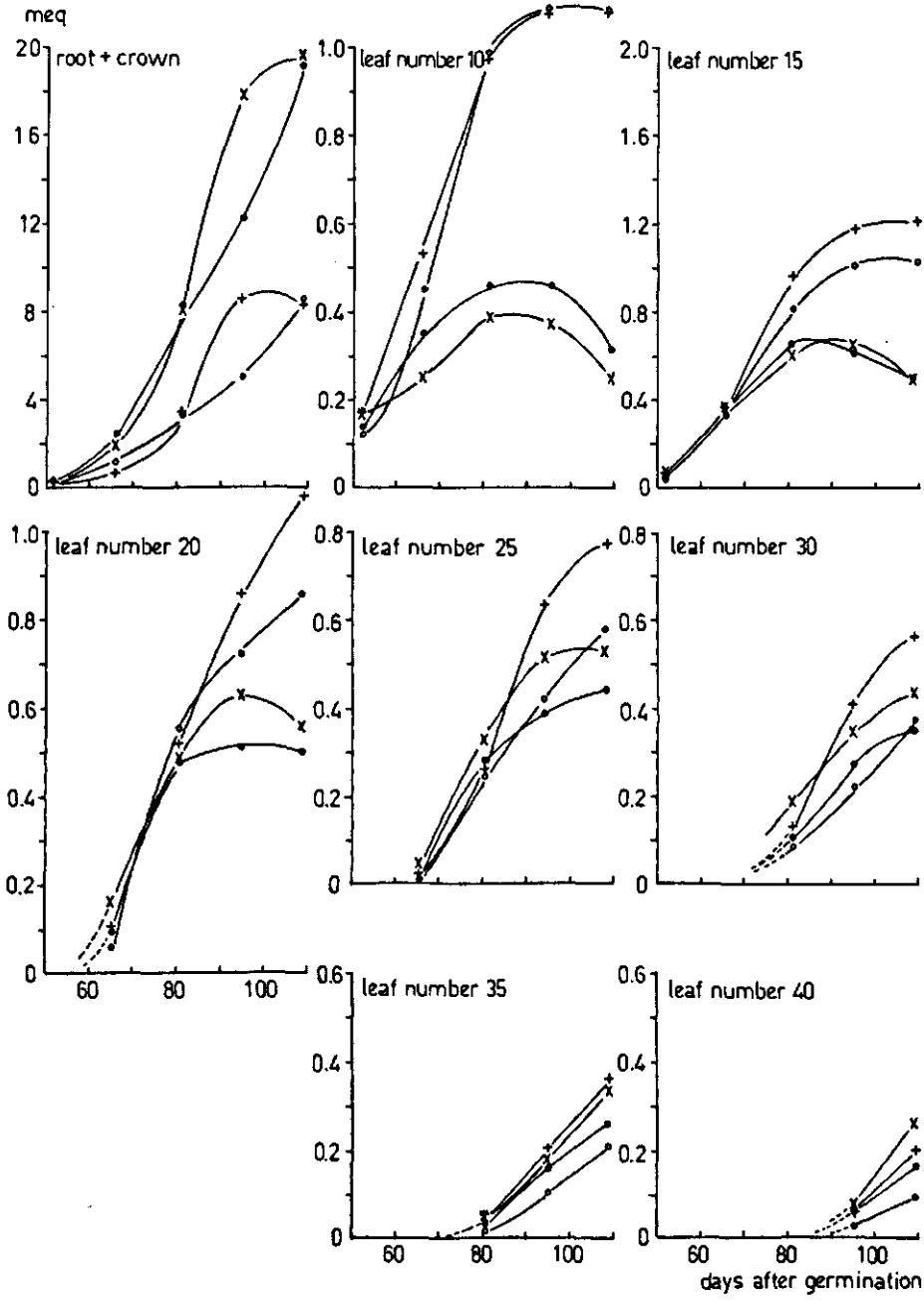


Figure 31. Amount of H_2PO_4 present in various plant parts and the amount of H_2PO_4 calculated with Cl as reference.

. = H_2PO_4
o = H_2PO_4 calculated } 60 kg N per ha

x = H_2PO_4
+ = H_2PO_4 calculated } 250 kg N per ha

clarified by some calculations.

The amount K redistributed towards root plus crown at 109 days is 28 and 57 meq per plant for the low and high N treatment, respectively. If all is potassium carboxylate, if these carboxylates are decarboxylated in root plus crown and if the anions set free are deposited with Ca and Mg in the xylem liquid, there is transported towards the leaves:

- low N treatment ΣC 388, ΣA 360 and (OH^-, HCO_3^- or CO_3^{2-}) 28 meq per plant
- high N treatment ΣC 513, ΣA 456 and (OH^-, HCO_3^- or CO_3^{2-}) 57 meq per plant.

If for every kg dry matter produced 200 litre is transpired (de Wit, 1958), the ions are transported in 0.38 x 200 = 76 litre and 0.43 x 200 = 86 litre of water, respectively. So average concentrations in the xylem liquid are:

- low N treatment ΣC $\pm$ 5.1, ΣA $\pm$ 4.7, (OH^-, HCO_3^- or CO_3^{2-}) $\pm$ 0.4 meq per litre
- high N treatment ΣC $\pm$ 6.0, ΣA $\pm$ 5.3, (OH^-, HCO_3^- or CO_3^{2-}) $\pm$ 0.7 meq per litre.

The acitivity coefficient at these concentrations is roughly 0.9 for univalent and about 0.75 for divalent ions.

If the total concentration of hydroxyl, bicarbonate and carbonate ions is about 0.55 meq per litre (the average of the two calculated values) and pH $\leq$ 8.5 the bicarbonate activity is approximately: $(HCO_3^-) = 0.9 \times 0.55 \times 10^{-3} = \pm 0.5 \times 10^{-3}$ eq per litre, while (CO_3^{2-}) and (OH^-) may be neglected. At 25°C $(H^+) = 10^{-7.81} \times CO_2(g)/(HCO_3^-)$, in which $CO_2(g)$ is the partial CO_2 pressure in the air.

In this case $(H^+) = 10^{-7.81} \times CO_2(g)/(0.5 \times 10^{-3}) = 10^{-4.51} \times CO_2(g)$, or $pH = 4.51 - \log CO_2(g)$. At $CO_2(g) = 0.0003$ atm, the normal partial CO_2 pressure in the air: $pH = 4.51 - \log 10^{-3.52} = 8.03$. The measured pH will be lower as the xylem liquid contains phosphate and the partial CO_2 pressure may be higher. The carbon dioxide effect on plant juices is about 1 to 2 pH units (Small, 1946). This calculation proves that in the case of sugar-beet sufficient bicarbonates may occur in a neutral to slightly alkaline xylem liquid, to provide the anions that are necessary for the redistribution of Ca and Mg.

The accumulation of redistributed K in the root and of redistributed Ca and Mg in the old leaves is still unexplained, but it is expected that the latter cations accumulate in organs with an overproduction of oxalate. The ratio

$$\{(A_{Cl}/U_{Cl})(U_{Ca} + U_{Mg})\}/Ox \qquad (2)$$

is a measure for overproduction (<1) or underproduction (>1) of oxalate. A is the amount of the indicated ion in the organ in meq, U is the amount in the whole plant and Ox is the amount meq of oxalate produced in the organ.

Table 11. Redistribution of Ca or Mg. High N treatment at 95 days.

Organ	Redistribution of Ca or Mg	Over- or underproduction of oxalate according to Eqn(2)
crown + root	from	1.26
leaf no. 10	towards	0.70
15	towards	0.92
20	towards	0.94
25	?	1.10
30	from	1.30
35	from	1.42
40	from	1.52

From Figs. 29 and 30 it is seen that for the high N treatment at 95 days the redistribution of Ca and Mg is most obvious. For this case it is shown in Table 11 that Ca or Mg are redistributed from organs with underproduction towards organs with overproduction of oxalate.

In the first part of this section the assumption is made, that the xylem liquid has a positive (C-A) value. If this assumption is correct the redistributing Ca and Mg already enters the leaf accompanied by bicarbonate or by carboxylates. In the roots enough malate and citrate is available for such transport. The presence of carboxylates in the xylem liquid is mentioned by Stewart (1963).

6 Root quality

The quality of sugar-beet roots is to some extent determined by physical characteristics, but mainly by the sugar, sodium, potassium and α-amino or noxious nitrogen content. These chemical characteristics are markedly affected by fertilizers.

The proportion of the sucrose extracted by the factory process (some is lost because it remains uncrystallized in the molasses) depends on the juice purity, which expresses the weight of sugar as a percentage of the total weight of dissolved solids present in the beet juice. Carruthers & Oldfield developed formulae by which the juice purity can be calculated and Ludecke developed formulae for the Zuckerausbeute. For example: Juice purity = 97.0 - 0.0008 (2.5 K + 3.5 Na + 10 α-amino N), K, Na and N being expressed as mg per 100 gram sugar.

In Section 5.4 it is shown, that (a) after the month of July large amounts of organic N are redistributed towards the roots, (b) the young leaves of the low N treatment import a great deal of their organic N from the old leaves, (c) in the high N treatment root plus crown reduce after July considerably more nitrate than in the low N treatment (in September even 10 times as much) and the carboxylate slag in the root plus crown increases proportionally. Therefore root quality is favoured by such an amount of nitrogen fertilizer, that the plants exhaust the nitrate stock during August. Thus less nitrate is reduced and less carbohydrates consumed for nitrate reduction and consequently more carbohydrates are available for storage in the roots. Moreover, the depletion of the nitrate stock restricts the carboxylate slag in the roots, while due to organic-N redistribution the roots do not starve for nitrogen. The reverse of these effects may be expected when nitrogen is top-dressed in June or July and for that very reason such top-dressing can have a decreasing effect on the quality of the roots.

Neither the total amount of cations nor sodium are redistributed and the redistribution of potassium is counterbalanced by calcium and magnesium (see Section 5.5). Van Egmond (1971) showed with data of Draycott et al. and of Rosanow that application of sodium increases the amount of potassium in the

root section of the sugar-beet crop by about 10% and the percentage of potassium in the plant that is found in the roots up to about 28%. The last fact can be related to a relatively higher potassium redistribution from the leaves of high Na/K - than of low Na/K - treatments as follows: when there is between the treatments little difference in the amount of nitrate reduced in the leaves, the amount of carboxylates (mainly oxalates) in the leaves will be about the same in all treatments (see Section 5.3). About absolutely equal amounts of potassium will then be redistributed from the leaves to the roots, as this redistribution is linked to the overproduction of oxalates (see Section 5.7) in the leaves. This means that from the relatively small amount of potassium in the leaves of the high Na/K treatment relatively much is redistributed towards the roots.

As Na does not redistribute it follows from the foregoing that adequate dressings of low percental potassium fertilizer rather than high percentual increase the amount of sodium in the root and do not decrease that of potassium. Therefore, the quality of the ash is negatively influenced. The juice purity decreases according to the formula given in this section by about 0.32 and 0.39 units as the amount of Na and K, respectively, increases with 5 meq per 100 gram sugar. However, under normal manuring conditions, altering the cation composition of the fertilizers is not likely to improve the quality of the roots for three reasons.

Firstly because the ash composition is determined by the ratio between the cations that are taken up and to a large extent by redistribution of the cations. From the potassium concentration in the roots and the percentages of redistributed potassium given in Section 5.5, it follows that potassium redistribution raises the potassium content in the roots by 100 to 200 meg K per kg dry matter. When 2/3 of the dry matter is sugar, this means a potassium increase of 15 to 30 meq per 100 grams of sugar. The negative influence on juice purity of these increases could be 1.17 to 3.34 units.

Secondly because in the Netherlands the potassium status of the soils on which sugar-beets are grown is sufficient.

Thirdly because potassium manuring increases the sugar content of the roots slightly but consistently (Draycott & Cooke, 1966).

The photosynthetic acitivity of the oldest sugar-beet leaves is low*; more-

*personal communication of L.J.P. Kupers regarding the research of J.P. van Loon (1972) (ALG 265).

over, these leaves are in a closed crop heavily shaded. Therefore a decrease in potassium redistribution effected by forcing the loss of old leaves might influence the quality of the roots positively. As organic N and phosphate as well as potassium are redistributed from the old leaves, the loss should not be forced when the plants have depleted the nitrate stock in August. But for those plants with a still ample supply of nitrate in August, forced loss of old leaves in August and thereby a decrease in potassium and organic-N redistribution seems a suitable means to improve the quality of the roots. At the end of the growing period about 1 meq K per plant per day is redistributed from the leaves - mainly the 15 oldest - towards the root (Fig. 28). If it is assumed that the potassium redistribution can be diminished by ½ meq K per plant per day for 45 days by removing the 15 oldest leaves from the plant in the middle of August, the redistribution of potassium towards the root is 22.5 meq less. Thereby the potassium content will be about 100 meq per kg dry matter lower or the juice purity about 1.17 higher. As per unit decrease in juice purity about 1.75% of the sugar is lost during manufacture, the decrease in K redistribution already means that 2% more sugar is produced. Moreover, the organic-N redistribution towards the root could decrease by about 70 mmol per plant, which is derived from Figs. 24 and 25. However, mechanical defoliation of old leaves, cannot be practised in August without seriously damaging the sugar-beet crop.

What other cultivation practices for forcing the loss of old leaves should be considered? Generally spoken those that force the loss but do not accelerate the redistribution from the old leaves. That the redistribution of the alkaline earths from the roots might thereby be decreased too is not harmful as these ions are removed during the purification of the juice in the factory.

Introduction of yellow wilt by lice in the sugar-beet crop in August especially forces the loss of old leaves, but it is unknown whether redistribution is not accelerated at the same time and photosynthesis is negatively influenced (Hall & Loomis, 1972). In comparison with corresponding controls a significant decrease of quality of virus-infected beets has been demonstrated (Burba, 1971). About 25% more molasses was produced, because of the higher potassium, sodium, amino-N and betaine content of the virus-infected beets.

Spraying chemicals that force the loss of old leaves or decrease potassium and/or organic-N redistribution from these leaves might be a suitable method, but little is known of the effect on sugar-beet root quantity and quality. Humphries & French (1965) established that (2-chloroethyl)trimethylammonium chloride increases while gibberellic acid decreases the number of

leaves produced by sugar-beet plants, but a relatively equal amount of leaves had died in each treatment by the end of October. There have been reports (Amchem, 1969) that Ethrel applications increased root yield but decreased sugar content and could increase the total amount of recoverable sugar by approximately 5%.

Research focussed on the influence of forced 'loss' of old leaves on quantity and quality of sugar-beet crops overdosed with nitrogen are of practical interest as every % gain in sugar percentage means about 500 kg per ha extra sugar production and every unit gain in juice purity means about 875 kg per ha extra sugar recovery.

Kupers (pers. communication) as well as Houba (pers. communication) already started research on the influence of loss of old leaves. It seems worthwhile to continue and extend this research, in order to translate the theoretical possibilities into applicable cultivation practices that improve root quality.

7 Summary and conclusions

Generally there is no paired uptake of C and A from neutral salts. Acidic as well as alkaline uptake processes occur, in which carboxylates are involved (see Section 2.1.2). However, sugar-beet plants do take up almost stoichiometrically equal amounts of C and A (see Table 10, Section 4.3). Houba et al. (1971) and Houba (1973) established that C and A are taken up stoichiometrically equal as long as the nitrate supply suffices, otherwise an alkaline uptake process (no. 2 of Table 1) sets in. In about 100 days the nitrate stock in this low N treatment was depleted, but alkaline uptake was not observed (see Table 10). Possibly the period of nitrogen shortage was too short, or the ageing sugar-beet plant lost the capability of starting the alkaline uptake.

The excess of charge represented by (C-A) is neutralized by organic anions. The equality between (C-A) and sum of carboxylates is confirmed (see Section 4.3). The amount of carboxylates is larger than that of inorganic salts and oxalate is the predominant anion (see Fig. 13).

The oxalate and the sum of Na + Ca + Mg accumulate almost stoichiometrically in the plant (see Fig. 14). Because of the low solubility product of certain oxalates, crystals are formed. Lots of crystals are observed in cuttings of fresh sugar-beet leaf material (see Fig. 15).

The extent of the carboxylate pool is determined by the nitrate reduction (see Table 10). Obviously, the influence of the sulphate reduction on the carboxylate pool and other influences, such as loss of leaf material, sampling and analytical errors are relatively small and compensate each other.

Large changes in pH of the cell solution due to ion uptake and utilization are prevented by changes in the carboxylate pool, besides the accumulation of carboxylates increases the buffer capacity.

The question - which (C-A) content for sugar-beet is the 'normal' one - remains. In fact in this study the relation between (C-A) and yield is a pseudo relation, derived from the relation organic N and dry matter production.

In general it is impossible to estimate quantitatively import, export, etc. by analysing time courses of accumulation only. Yet for sugar-beet it is possible as in Section 5.3 it is shown that Cl, which accumulates monotonically in

the leaves, is a good index element.

The sum of NO_3 + (C-A) in the various organs are equal to the calculated nitrate supply (see Section 5.3), so that neither chloride nor nitrate are redistributed. This proves that nearly all carboxylate formed during organic-N production from nitrate and organic compounds stays at the place where it is formed. As the amount of carboxylates in root plus crown is relatively low, an important consequence of these conclusions is that nitrate is mainly reduced in the leaves and only to some extent in root plus crown.

The redistribution of organic N, as gathered from the organic N and (C-A) data is described in Section 5.4. The organic N is redistributed from ageing leaves towards young leaves and root plus crown. At 109 days about half of the organic N present in root plus crown is redistributed organic N.

At the end of the growing period, the growth of the young leaves in the low N treatment depends on the part of the redistributed organic N that reaches these leaves, while at the same time in the high N treatment the young leaves are supplied with nitrate and become self-supporting for organic N.

In Sections 5.5 and 5.6, it is shown that the effect of redistribution on the total amount of positive and negative ions present in the various organs is nil. Potassium and phosphate are redistributed from old leaves towards young leaves and root plus crown; calcium and magnesium from root plus crown towards the old leaves; sodium, chloride, nitrate and sulphate are not redistributed. These conclusions are limited because leaf blade and petiole or root and crown are not separately treated (see Section 5.2).

The redistributing calcium and magnesium from root plus crown towards the old leaves can be transported via the xylem. In Section 5.5 an explanation is given as to why this redistribution is less easily demonstrated than that of potassium. The limited Ca redistribution from the leaves is probably not due to the inability of Ca to move in the phloem but to a great accumulation and absorption capacity of the various leaf tissues. The accumulation of carboxyaltes in especially the old leaves agrees well with this assumption.

A small amount of anions suffices to accompany the redistributing potassium which is counterbalanced by calcium and magnesium redistribution. Inorganic anions are hardly redistributed (see Section 5.6). In Section 5.7 it is suggested that potassium carboxylates are redistributed from the old leaves via the phloem and that calcium and magnesium bicarbonates or carboxylates are redistributed from root plus crown via the xylem.

It is shown in Section 5.7 that calcium or magnesium are redistributed from organs with underproduction towards organs with overproduction of oxalates

(see Table 11). An equation is given by which the overproduction and underproduction of oxalate can be calculated.

In Chapter 6 the results are discussed with respect to the quality of sugar-beet roots.

Depletion by the plants of the nitrate stock during August restricts the carboxylate slag in the roots, while due to redistribution of organic N the roots do not starve for nitrogen (Section 5.4). Moreover, the less carbohydrates are used for nitrate reduction, the more they are available for storage in the roots. Therefore it is concluded, that depletion of the nitrate stock during August favours root quality.

Although, in Chapter 6 it is explained why low percentual potassium fertilizer reduces the 'quality' of the ash, it is concluded for a number of reasons that in the Netherlands, application of fertilizers with different ratios between sodium and potassium is unlikely to improve the quality of the roots.

Redistribution from old leaves raises the potassium content in the roots by 100 - 200 meq K per kg dry matter (Section 5.5). Therefore forced loss of old leaves might influence the quality of the roots positively. When the redistribution of potassium can be diminished by a factor 2 for 45 days about 2% more sugar might be produced in the factory.

Mechanical defoliation of old leaves cannot be practised in August without seriously damaging the sugar-beet crop. Too little is known of the effect on root quantity and quality of spraying chemicals that force the loss of old leaves or in another way decrease potassium or organic-N redistribution. Therefore, how interesting defoliation is theoretically, at the moment no practically applicable method of it can be given.

Samenvatting

In een veldproef met twee stikstofniveaus werd de ionenbalans van de suikerbietenplant bestudeerd. De ionenopname werd gemeten door op zes tijdstippen gedurende het groeiseizoen de chemische samenstelling van de plant te bepalen. Zowel de ionenverwerking als de distributie en redistributie werden bestudeerd aan de hand van de droge-stofopbrengsten en de chemische samenstelling van de diverse bladeren en de biet.

De suikerbietenplant neemt, in tegenstelling tot bijvoorbeeld de Gramineae, vrijwel stoechiometrisch gelijke hoeveelheden C en A op. Eerder werd vastgesteld, dat bij onvoldoende nitraataanvoer de opname van A achterblijft bij die van C. Echter ook in de behandeling waar de nitraatvoorraad na 100 dagen uitgeput was, werd geen 'alkalische opname' waargenomen. De mogelijkheid wordt geopperd, dat hiervoor de periode van stikstofgebrek te kort is geweest of, dat oudere planten het vermogen om het 'alkalische opname'-proces te starten verloren hebben.

De gelijkheid tussen het verschil van anorganische kationen en anionen: de (C-A) en de som van de carboxylaten werd bevestigd. De hoeveelheid carboxylaat is groter dan de hoeveelheid anorganische zouten en oxalaat is het anion dat het meest in de suikerbietenplant voorkomt. De oxalaationen en de (Na + Ca + Mg) ionen accumuleren in vrijwel stoechiometrisch gelijke hoeveelheden in de plant.

De nitraatreductie bepaalt de grootte van de carboxylaatvoorraad. Klaarblijkelijk zijn de andere invloeden (waaronder de sulfaatreductie, het bladverlies, de monstername-fout en analytische fouten) relatief gering en compenseren zij elkaar. De carboxylaten kunnen dan ook opgevat worden als een slak die zich vormt tijdens het nitraatreductie-proces.

De resultaten bevestigen dat grote pH-wijzigingen tengevolge van de ionenopname en ionenverwerkingsprocessen worden voorkomen door wijzigingen in de carboxylaatvoorraad in de cel; bovendien vergroot een ophoping van carboxylaat de buffercapaciteit.

In het algemeen kan de import, export, etc. van diverse organen niet kwantitatief bepaald worden door de accumulatie gedurende het groeiseizoen te

analyseren. In het geval van de suikerbiet blijkt dit echter wel mogelijk, daar aangetoond werd, dat Cl een goede referentiebasis is voor de berekening van de aanvoer van andere ionen.

De geaccumuleerde hoeveelheden $\{NO_3 + (C-A)\}$ in de diverse organen zijn gelijk aan de berekende hoeveelheden aangevoerd nitraat. Dit betekent, dat de carboxylaten dáár blijven, waar ze tijdens het nitraatreductie-proces worden gevormd. Omdat de hoeveelheid carboxylaat in de biet relatief gering is, volgt hieruit dat het nitraat hoofdzakelijk in het blad en slechts in beperkte mate in de biet wordt gereduceerd.

Het verschil tussen de hoeveelheden (C-A) en organisch stikstof in een orgaan is een maat voor de redistributie van organisch stikstof. Het organisch stikstof wordt geredistribueerd van de oude bladeren naar de jonge bladeren en de biet.

Het effect van de redistributie op de totale hoeveelheid positieve en negatieve ionen, aanwezig in de diverse organen, is nihil. Fosfaat en kalium worden geredistribueerd van de oude bladeren naar de jonge bladeren en de biet, calcium en magnesium van de biet naar de oude bladeren, terwijl natrium, chloride, nitraat en sulfaat niet geredistribueerd worden. Verondersteld wordt, dat kaliumcarboxylaat de oude bladeren via het floëem verlaat en dat calcium- en magnesiumbicarbonaat en/of carboxylaat uit de biet via het xyleem wordt geredistribueerd. De redistributie van calcium en magnesium vindt plaats van organen met een relatief geringe, naar organen met een relatief grote oxalaatproduktie.

Door uitputting van de nitraatvoorraad door de planten in augustus blijft de carboxylaatslak in de biet beperkt, terwijl door redistributie van organisch stikstof de biet toch voldoende stikstofaanvoer heeft. In dit geval worden ook minder koolhydraten gebruikt voor de nitraatreductie en zijn er meer koolhydraten beschikbaar voor opslag in de wortels. Daarom wordt er geconcludeerd, dat uitputting van de nitraatvoorraad in augustus de kwaliteit van de bieten bevordert.

Redistributie van kalium uit de oude bladeren kan het kaliumgehalte van de biet met 100 tot 200 meq per kg droge stof verhogen. Geforceerd verlies van de oude bladeren zal de kwaliteit van de biet mogelijk gunstig beïnvloeden. Wordt de redistributie van kalium in de laatste 45 dagen van het groeiseizoen bijvoorbeeld tot de helft teruggebracht, dan kan in de fabriek ongeveer 2% meer suiker geproduceerd worden.

Het lijkt zinvol het onderzoek te continueren en daarbij te proberen de theoretische mogelijkheden te vertalen in praktische cultuurmaatregelen die de kwaliteit van de bieten bevorderen.

Appendix

CARBOXYLATE DETERMINATION

In Fig. 32 a scheme is given of the carboxylate determination.

A subsample of 250 - 500 mg plant material, dependent on the expected carboxylate content, is put in the extraction vessel with 10 ml H^+ sulphonic acid resin (Merck no. 1) and 50 ml deionized water. The mixture is stirred for 10 minutes. By opening the stopcock of the extraction vessel a clear extract drains into an adjoining conical flask, as floating particles are stopped by the wadding. The stopcock is closed again just before all extract is drained from the mixture. The extraction procedure is repeated 7 times.

After addition of 0.5 ml 0.5 N H_2SO_4 and 0.5 gram silica (Merck 0.05 - 0.2 mm) the extract is evaporated to dryness by 30 - 35°C in a rotating vacuum evaporator (Büchi). The coated silica is removed from the evaporating flask and stored in a test tube. The remainder in the evaporating flask is dissolved in about 4 ml deionized water and after addition of 0.5 gram silica (Merck 0.05 - 0.2 mm) again evaporated to dryness. The remaining coated silica is added to the silica already in the test tube. Closed test tubes are stored in a refrigerator.

In a mortar 4 grams of silica (Mallinckrodt 100 mesh) is stirred with 2.8 ml 0.5 N H_2SO_4. After suspending the silica in 20 ml chloroform the suspension is transmitted into a glass column. This column, whose end is plugged with glass wool, has been previously partially filled with chloroform. By opening the stopcock at the end of the column the superfluous chloroform drains away, while air bubbles are removed by stirring the suspension in the column with a glass rod. The package of the column is improved by raising the air pressure on top of the column. After addition of some ml of chloroform on top of the column a sample coated silica is inserted. Air bubbles are again removed.

In a vessel of 500 ml is deposited, a teflon coated magnetic rod, 300 ml chloroform and sufficient 0.5 N H_2SO_4 to remove all air from the vessel. The end of a pear-shaped funnel, with closed stopcock, is introduced into the vessel. The funnel should end in the chloroform and is fixed with a perforated

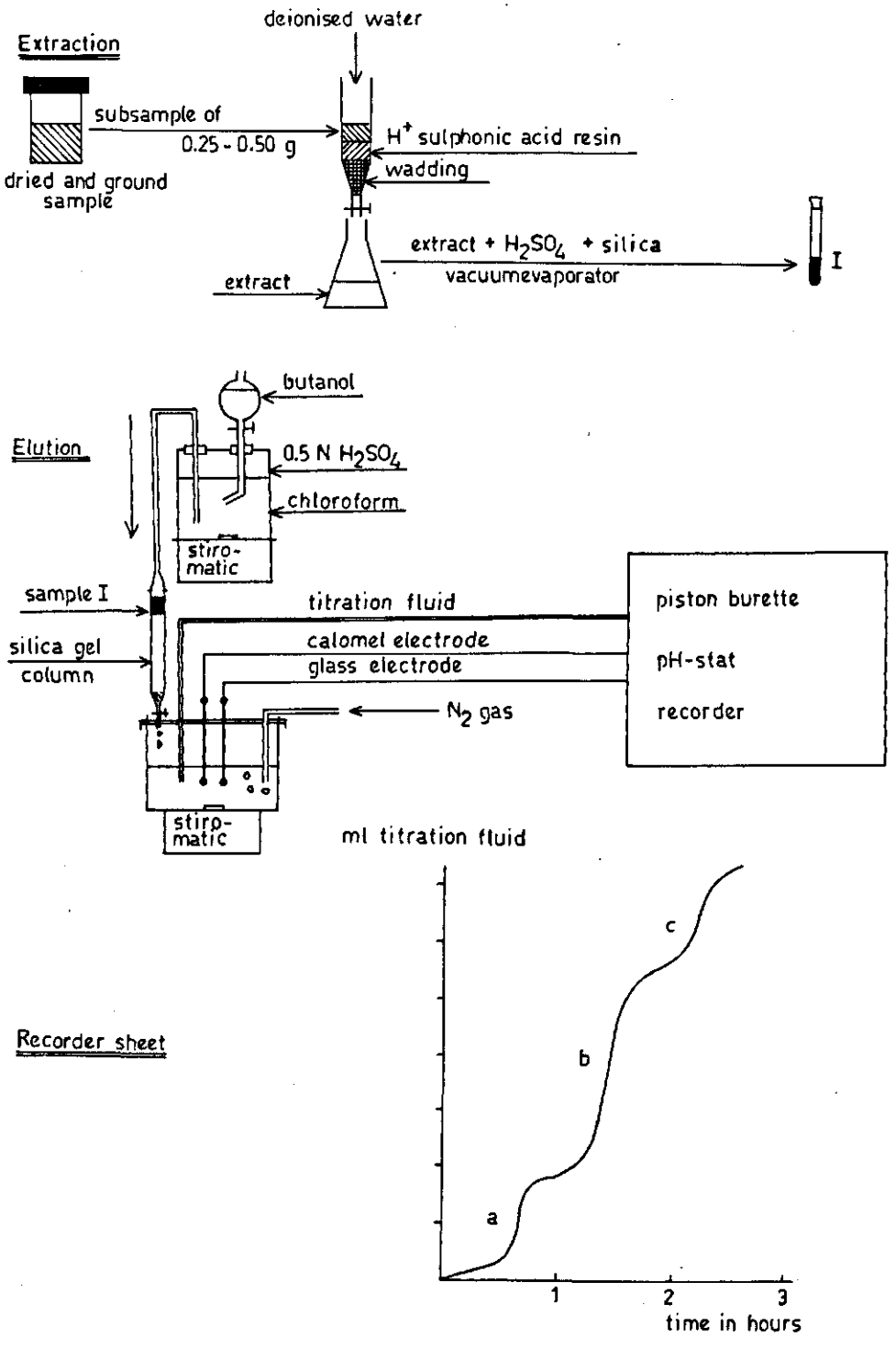


Figure 32. Scheme of carboxylate determination.

rubber stopper. A small amount of chloroform is put in the funnel, after which the air beneath the stopcock of the funnel is removed. The stopcock is closed when nearly all chloroform has passed. The ball and cup of the spherical joint of the siphon and the column are greased with Dowex High Vacuum Grease. The other end of the siphon is inserted into the vessel and fixed with a perforated rubber stopper. Air bubbles in the vessel are not allowed! Opening the stopcock of the pear-shaped funnel for a while results in cleaning the siphon of residues of H_2SO_4 by chloroform. The vessel is set on a magnetic-stirrer. The ball joint of the siphon is fixed with a clip to the cup joint of the column loaded with the sample. The pear-shaped funnel is filled with 175 ml 1-butanol.

The end of the column is inserted into a titration vessel through an opening in a loose cover. A teflon coated magnetic rod and about 200 ml deionized water are put in the titration vessel together with an alkali supply, a nitrogen gas supply, a calomel and a glass electrode. The alkali supply is connected to an automatic burette (S.B.U. 1b Radiometer) that is controlled by a pH-stat (T.T.T. 11 Radiometer) to which apparatus the electrodes are connected. The amount of alkali added to the titration-vessel is written by a recorder (syringe burette recorder type S.B.R. 2c Radiometer). The pH-stat is set on pH 8.5 (up scale) and the water in the titration vessel is titrated up to this pH. The titration vessel stands on a magnetic stirrer.

Table 12. Results of a series of organic acid analysis.

Component	Amount of acid in 10^{-6} eq			
fumaric acid	10.3	20.4	49.0	99.3
succinic acid	10.3	19.9	49.5	99.1
malonic acid	10.5	20.4	49.6	98.5
malic acid	9.9	19.4	49.5	98.3
citric acid	n.d.	19.4	49.0	99.0
tartaric acid	n.d.	n.d.	51.3	98.5
Total			297.9	592.7
Loading of silica sample per acid	10	20	50	100

n.d. = not detectable due to high background.

The analysis is started by switching on the magnetic stirrers and turning on the nitrogen gas supply as well as opening the stopcocks of the pear-shaped funnel and the column.

By gradient elution partition chromatography, the organic acids in the sample are separated over the silicagel column and thereafter automatically detected, titrated and recorded by the Radiometer set.

The analysis is stopped after the elution of oxalic acid to empty the titration vessel and to put in again 200 ml deionized water. Before the analysis is restarted the water in the titration vessel is titrated to pH 8.5.

A blank determination (the coated sample on the column has no organic acid loading) gives the background. The background increases with increasing butanol concentration in the elution liquid, as increasing amounts of H_2SO_4 per unit of elution liquid are deliverd into the titration vessel.

The amounts of the various acids are calculated as follows from the results of the recorder sheets:

$$X = (A - B)\, n$$

in which:

X = meq acid a (see Fig. 32),

A = ml alkali added to the titration vessel between, for example, 0 and 1 hour in the analysis,

B = ml alkali added to the titration vessel between, for example, 0 and 1 hour in the blank determination,

n = normality of the alkali used.

$$Y = (C-D)n - X$$

in which: X and n as before,

Y = meq acid b (see Fig. 32),

C = ml alkali added to the titration vessel between, for example, 0 and 2 hours in the analysis,

D = ml alkali added to the titration vessel between, for example, 0 and 2 hours in the blank determination.

NITRATE DISTURBANCE

Freeman (1967b) pointed out that high NO_3 contents in samples may cause too high values to be found when determining malate and citrate. A silica sample loaded with nitric acid disturbs the determination of oxalic but especially that of malic and citric acid (Freeman, 1967a). As the disturbance

is not equivalent to the amount of nitrate in the analysed plant samples, a correction based on the nitrate content is impossible. The disturbance takes place only when plant samples with very high nitrate contents, about 1000 meq NO_3 per kg dry matter, are analysed. The reason for this is that during evaporation of the acid extract of the plant material not all nitric acid is evaporated in the before mentioned cases. This problem can be solved by rewetting the coated silica (first sample) in the evaporation flask with 10 ml of deionized water and evaporating again to dryness, this procedure is repeated once.

RESULTS

In Table 12 results are given of a series of analyses in synthetic mixtures of organic acids.

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